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by:                      David Doyle, M.B., Ch.B. Edinburgh 1961.

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INTRODUCTION AND HISTORICAL REVIEW

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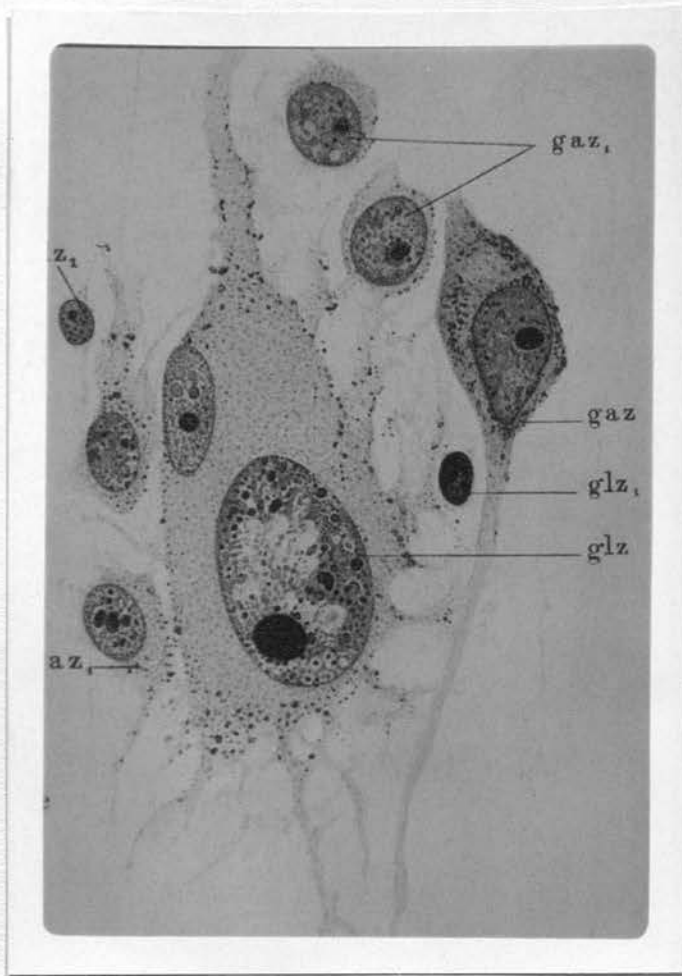


Fig. 1 Alzheimer's illustration of the type I giant-astrocyte (glz), with its large, hyperchromatic nucleus. Its size is compared with a smaller, normal glial cell (glz1) which is probably a microglial cell, and with two varieties of neurone (gaz, gaz1)

from von Höpflin u. Alzheimer.



This study began with a review of experimentally induced liver disease in rodents, with the objective of studying changes in the nervous system which might result. My introduction to the subject as a whole, and the reasons for the choice of porto-caval anastomosis in rodents have introductory bearing on my theme.

As a fourth-year student I saw a girl with hepato-lenticular degeneration undergoing porto-caval anastomosis for recurrent haemorrhage from oesophageal varices.

During my surgical residency in Professor Sir John Bruce's charge I saw a series of interesting patients:-

Mr. B., a 70 year-old, retired trades union official was investigated for jaundice, and found to have primary hepatic carcinoma. He passed through stages of euphoria, depression, and hallucination before dying in coma.

Mrs. C., a 50 year-old housewife, died after prolonged coma complicating biliary cirrhosis.

Mrs. K., an elderly, obese, jaundiced cirrhotic had a gastric transection (Tanner 1950) for bleeding varices, but died in coma.

For such altered mental states, for the confusions and comas occurring in those dying in uraemic coma, or in some who had had infusions of cancer chemotherapeutic substances into their carotid arteries I was given overlapping explanations, embracing the cerebral metabolism of amines, ammonia, urea and nucleic acids. In this nebulous biochemical field there seemed opportunities to do meaningful work.

A concept so wide required serious pruning. The literature was so involved and required such depth of knowledge that it took some time to achieve an understanding of the modern position, which was apparently as complete as technology would

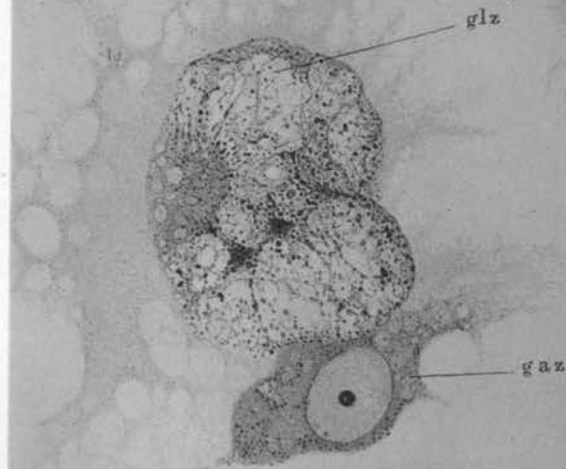


Fig. 1.

Fig. 2 An illustration of Alzheimer's type II giant astrocyte (glz), with its large, pale, indented nucleus. A neurone is shown for comparison (gaz).

from von Höpstin u. Alzheimer.

allow. There were two main avenues. One followed the acute hepatic encephalopathy; the other pursued the longer term neurological sequelae. I chose the latter because it seemed to involve more points of biological significance.

Hippocrates (460 - 356 B.C.) thought of the liver affecting mental activity, and wrote: "those who are made ..... on account of the bile are vociferous, vicious and do not keep quiet". This may not mean that he had seen hepatic encephalopathy, but may be an illustration of the ancient's understandable confusion with the functions of the parts of the body: Galen (150 - 199 A.D.) considered the liver to be the seat of the mind.

Morgagni, who founded descriptive morbid anatomy in the eighteenth century collected and correlated knowledge and writings up to his times. He found that it was then well-known that abnormal mental states, including coma, sometimes accompanied jaundice.

The histology of the brain received careful systematic attention for the first time during the technological renaissance at the junction of the 19th and 20th centuries. From the famous suburban Munich hospital, the Schwabing Hospital for Mental and Neurological Diseases (later called the Kraepelinen Institut), Alois Alzheimer, the foremost neurohistologist of the day published a paper with von Hoesslin about a young man who had died after a protracted illness with progressing dementia and neurological disability (1912). His clinical features included spasticity, ataxia, tremor, athetosis, and dysphasia - signs of widespread disease of the central nervous system. At the end of the case report they state that there was cirrhosis with a small liver, but they did not describe the cirrhosis.

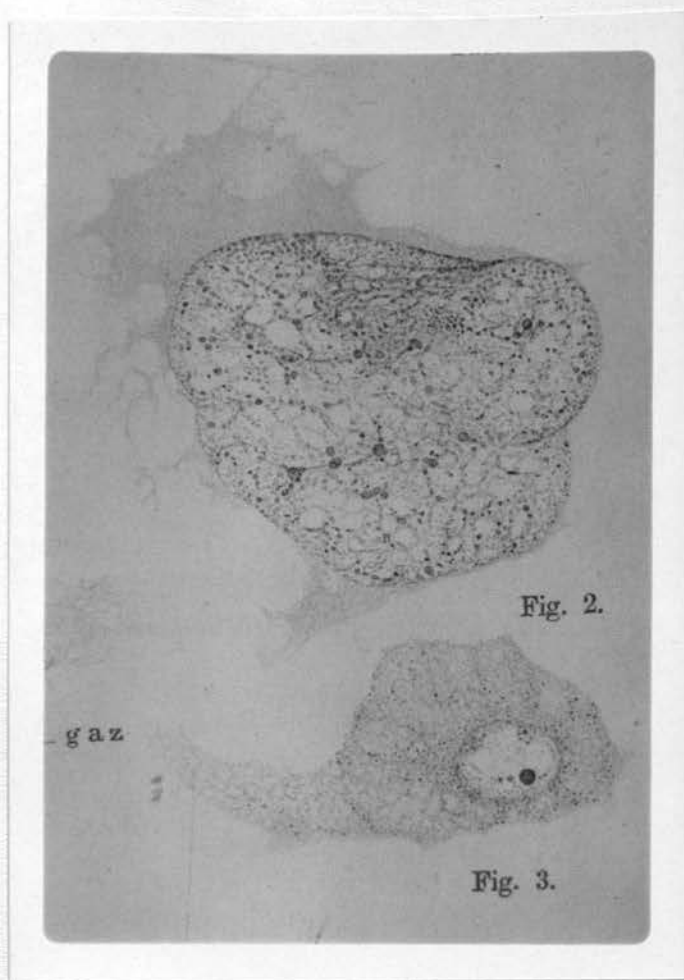


Fig. 3 An illustration of Alzheimer's type II giant astrocyte "Fig. 2.", with its large, pale, indented nucleus. A neurone is shown for comparison "Fig 3".

from von Höpflin u. Alzheimer.



The paper was "A Contribution on a Case of Westphal-Struempell's" Pseudosclerosis". Westphal (1883) and Struempell (1898) had already described the neurological features and the gross pathology of the firm, sclerotic, microcavitated brains in cases showing some of the clinical features of disseminated sclerosis. Alzheimer drew attention to a very important histological feature - the giant nuclei of astrocytes. Some of the nuclei were as large as the cell bodies of small neurones. Some had palely staining nucleoplasm and some were dark. These staining properties have been related to the quantity of DNA in the nuclei (Lapham 1963). Alzheimer called the pale cells type 2 and the dark ones type 1 (figs. 1 - 3). He also noticed apparently increased numbers of glial nuclei in many areas of grey matter.

S.A. Kinnear-Wilson (1912) described the clinical condition again, in greater detail, and with more cases from the neurological clinics of London. Two streams of thought then arose, one for unity and one for two apparently different disease entities - Westphal Struempell's Pseudosclerosis and Wilson's Disease or Hepato-Lenticular Degeneration. For many years contention raged until, eventually the neuropathological and clinical identity was established and the names of Wilson's Disease, or Hepato-Lenticular Degeneration survived.

Glazebrook (1946) and Cumings (1948) worked out the abnormality of copper metabolism in these people. Cumings found greatly increased quantities of copper in the brains and livers of people dying with this disease, and the reduction of circulating copper oxidase or caeruloplasmin was established. John Walshe (1956) studied the action of penicillamine and noted its satisfactory action in increasing enormously the urinary excretion of copper and the beneficial effect for the patients.

The similarity of the neuropathological features of hepato-lenticular degeneration to the findings in those dying after

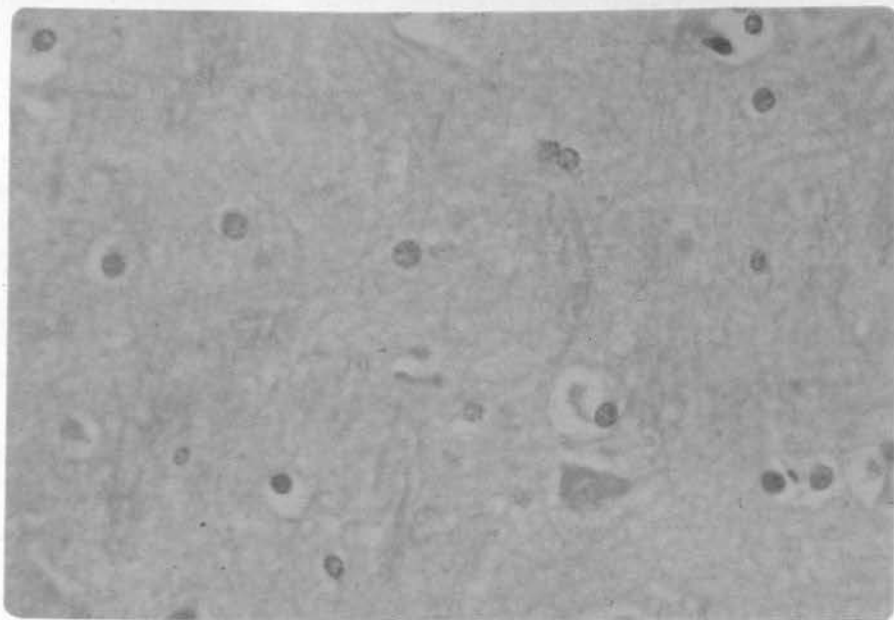


Fig. 4. A segment of normal dentate nucleus of a male aged 35. The astrocytes are of uniform size, staining properties, and circular outline (H-E x 750).

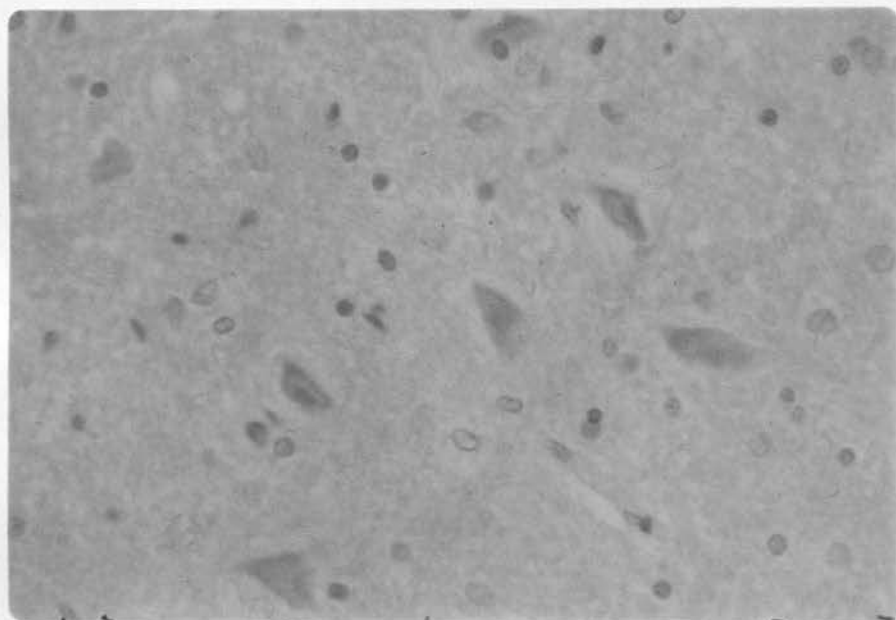


Fig. 5 A segment of dentate nucleus from a patient who died with portal cirrhosis. Encephalopathic features were not recorded in life. There is considerable variation in the size and shape of the nuclei of astrocytes, and the larger ones stain palely. The absence of stainable cytoplasm in some leads to their description as "naked nuclei" (H-E x 750).



years of cirrhotic or man-made porto-systemic anastomoses has been the subject of interest for many years.

In the early days, Spielmeyer (1922) had pointed out that Alzheimer's cells could be found in a variety of non-hepatic diseases not obviously related, and it is our day to day experience that type 2 cells occur frequently in uraemia, cachexia from carcinoma, and in advanced old age, although they are most frequently seen in cirrhotic liver disease.

There are no hard and fast criteria defining these cells in respect of size, shape or staining reaction. The gradation from a reacting astrocyte - a "progressive change" of Oscar Creutzfeldt - to the full blown Alzheimer type 2. nucleus is complete (figs. 4 - 8), as illustrated in preparations taken from the Neuropathological Laboratory in Edinburgh.

Other neuropathological features were described. Opalski (1930) described a large cell with a large cell body and clearly stainable nucleus. These cells, however, bear the characters one would now ascribe to some degenerate neurones, with shrunken, mottled cytoplasm and ill defined nuclear margins (Adams and Foley 1965). Opalski opined that they were phagocytes.

Neurones die in advanced hepato-lenticular degeneration (Adams and Foley 1953) and it is thought that the consequent loss of tissue leads to micro-cavitation or spongiform change in the cortex and basal ganglia. Reparative fibrous gliosis occurs but poorly in such areas, and proliferation of fibrillary astrocytes is not a feature in cases not affected by spongiform change. Reactive gliosis, or increase of the size and probably also of the numbers of protoplasmic astrocytic nuclei occurs at an earlier stage and may progress

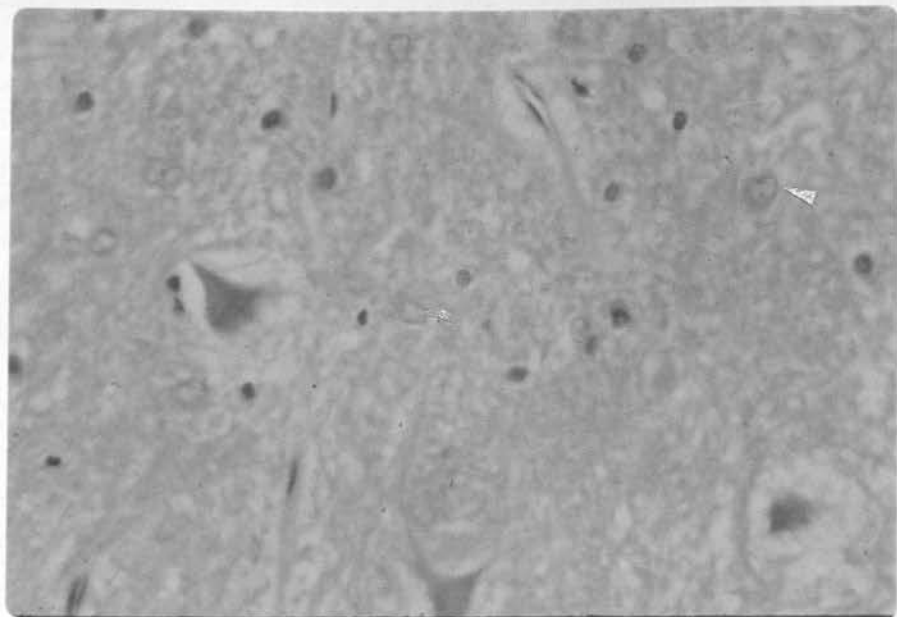


Fig. 6 A segment of dentate nucleus from a patient who had a porto-caval anastomosis for two years for the consequences of post-necrotic scarring. He died in coma. The astrocytic nuclei are larger and more irregular than in Fig 5. (H+E x 750).

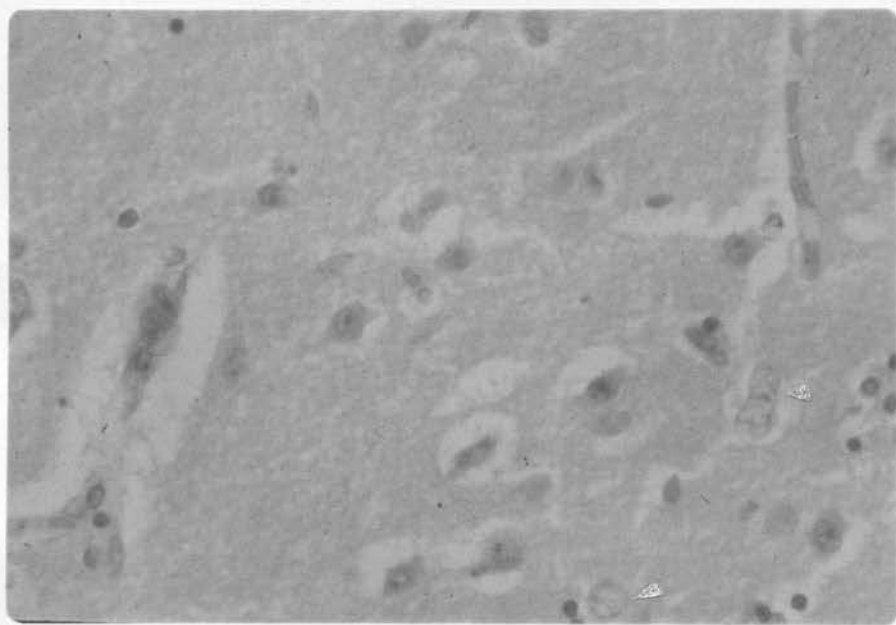


Fig. 7 A segment of dentate nucleus from a patient of 28yrs who died with Wilson's disease. Variation of size, shape and staining properties of the astrocytic nuclei is seen.

(H+E x 750).

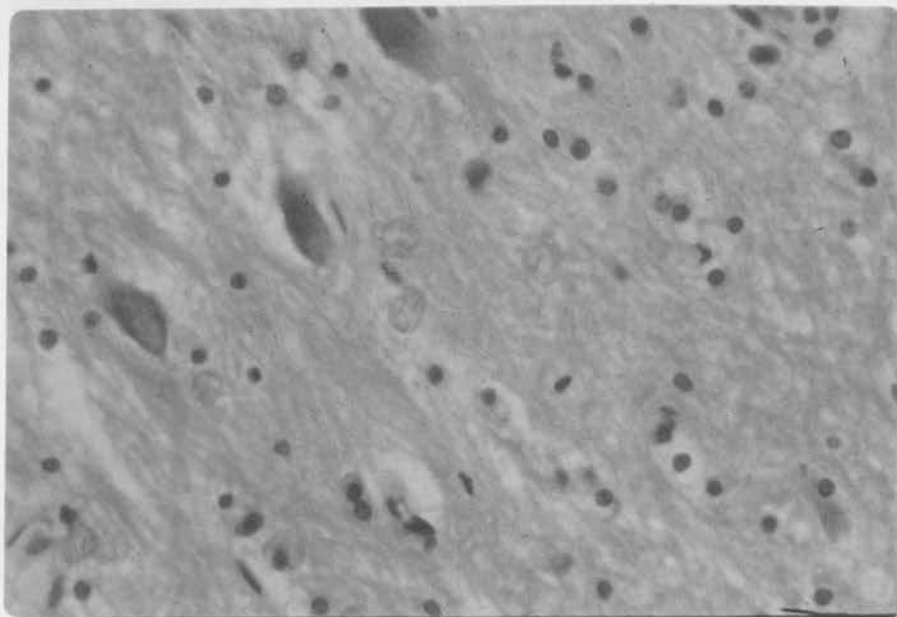


Fig. 8 A segment of dentate nucleus from a patient with post-necrotic cirrhosis who had a well-developed collateral portal-systemic venous drainage, who had had numerous episodes of stupor and haematemesis, and who died after being in coma for 5 days. The large size, pale staining, and irregularity of some astrocytic nuclei can be appreciated. Most of the other glial nuclei in this picture are oligodendrocytic.

(H.E. x 750).

with time so that progressive protoplasmic astrocytic and mild reparative fibrillary astrocytic changes eventually occur simultaneously.

These features are not confined to the basal ganglia, but occur throughout grey matter, or in relation to neurones anywhere - cortex, basal ganglia, dentate of cerebellum, brain stem and pontine nuclei and even in the spinal cord. So well developed do the changes in the astrocytes become in the dentate nucleus of the cerebellum that this easily sampled area may be used as a "screening test" to assess the likelihood of finding changes elsewhere in the post mortem brain.

In advanced cases of Wilson's disease, or in those neurologically altered by repeated episodes of hepatic stupor or coma, degeneration of long fibre tracts is seen, especially in cortico-spinal tracts and in the fibres around the periphery of the cord (Adams and Foley 1965). This may be degeneration consequent on loss of neurones at higher levels.

The similarity between the neuropathological findings in hepato-lenticular degeneration and in patients with fixed neurological disability following porto-systemic anastomosis - either man made or with liver disease - is remarkable. Adams and Foley have described the similarities in full and minted the title "Non-Wilsonian Hepato-lenticular Degeneration". The findings in their cases of Alzheimer's cells, Opalski cells - which they clearly trace from degenerating neurones - and of long-tract degeneration are the same as in the classical Wilsonian syndrome. Clinically the degeneration of neurological function with liver disease or porto-caval anastomosis runs a similar course to Wilson's disease. Differences are that Wilsonian subjects are more youthful, and in the non-Wilsonian group there is neither familial pattern nor abnormality of copper metabolism.



Taking a standpoint such as this, that there is virtually complete identity between the groups, it is easy to postulate why the world's literature carries so many cases labelled as Wilson's disease which fail to show any abnormality of copper metabolism or which have failed to improve on penicillamine therapy.

There is a danger in tending too far towards unification, especially when dealing with histopathology in the nervous system where the repertoire of the various cellular elements, in response to disease, is limited. But known facts point towards unity of histology and it is tempting to suggest that the causes may be the same. Liver disease with consequent porto-systemic shunting or surgical porto-caval anastomosis may be the common factors in all these cases.

When I began this study, I found the work of Alzheimer, Spielmeyer, Opalski, and Adams and Foley, but found very few references to animal experimentation, surprisingly for a problem with such apparently wide implications. If ever there was an avenue leading to the study of biochemical and histological variations in neurological disease, this was it.

Meat intoxication was shown in dogs with Eck's fistulae by Pavlov (1923) and its counterpart, the stupor of man following digestion of blood in the alimentary tract had long been known - Sherlock (1965). Changes in the astrocytes of such dogs' brains had also been shown (Kline 1965). There were no studies of the brains of rodents.

The appeal of the rodent was the appeal of controllable conditions. Rats were also cheap and were bred in the department.

How to produce an experimental model was difficult to see. The systemic administration of powerful poisons such as carbon tetrachloride, yellow phosphorus, allyl formate or bromobenzene bore the hazard of their possible direct effects on the brain. Special diets were too expensive and difficult to manage.

An experiment was designed to produce a colony of cirrhotic rats using carbon tetrachloride by injection intraperitoneally. The dosage schedule was suggested by Dr. Patrick of Glasgow University. The experiment failed because of my catching influenza after six weeks. The cessation of injection of the carbon tetrachloride allowed the livers to return to normality. A publication at that time (Lapham 1963) decided me against resumption of that work. Lapham stated that he had produced cirrhosis in rats using carbon tetrachloride, and found both types of Alzheimer's cells in the brains from four weeks after the commencement of injections. He used ultraviolet microspectrofluorimetry and showed a gradation of nuclear DNA content between normal and hyperchromatic type 1 nuclei. The DNA complement rose from a normal diploid to tetraploid or higher polyploidy and he deduced evidence for preparation for, and subsequent division of astrocytes by amitosis.

It had been shown that rats with experimentally induced cirrhosis have portal hypertension and develop porto-systemic anastomoses (Bono 1960) in the same way as humans with portal hypertension. The operation of porto-caval anastomosis seemed the "cleanest" and most effective way of producing what seemed to be the possible definitive abnormality - porto-systemic shunting of blood.

Professor Sheila Sherlock advised me that I should not pin any hopes on the success of the operation in rats but also



advised that whatever model I used, I should compare it as closely as possible to disease in man: blood ammonia estimations, she said, would have to be done whatever the model.

The development of the operation is included as part of this thesis. The work was full of instruction in the principles of experimental surgery and anaesthesia, for I had no instruction or assistance.

The estimation of blood ammonia by Conway's classic method was tried but proved beyond my resources of space, time and money. It would have taken too long to produce consistency, and no-one else locally had the apparatus in use. This method was eventually abandoned in favour of the successful ion-exchange method of J.C.B. Fenton. The method was brought to my notice by Dr. Tompsett, Reader in Clinical Chemistry in Edinburgh. His department kindly gave me space and apparatus. Having found a good experimental model and a way of comparing it with known facts in man - namely the ammonia intoxication theory of encephalopathy (in Sherlock 1965). I had to know what to look for in the histological material. Dr. A.F.J. Maloney coached me in the changes in human brains. Dr. J.B. Cavanagh had given me some advice on how to handle the problem in rats, and had referred me to the technique of autoradiography for the study of dividing cells.

Many questions seemed worthy of consideration apart from the cerebral histology.

What would happen to the liver itself?

What would the kidneys do in the face of the altered arterial perfusion?

Would the stomach show ulceration as occurred sometimes in man?

These and other questions I have attempted to answer, but the limitations of one man working alone have necessitated simple techniques and simple reasoning. Some answers have appeared, but many more remained unanswered. Some of the problems have already been taken up by others.

In Summary, the contents of this thesis comprise:-

1. Establishment of the Experimental Model - the Porto-caval Anastomosis in rats.
2. Studies of the histology of liver, brain, kidneys, adrenals and stomach.
3. Autoradiographic (tritium-labelled thymidine) studies of all tissues, but with emphasis on liver, brain and kidney.
4. Plasma ammonium ion determination.
5. Brain Biochemistry - some preliminary studies.

THE PORTO-CAVAL ANASTOMOSIS - ECK'S FISTULA

Ligation of the portal vein in quadrupeds had long been known to have a fatal consequence when Schiff (1877 and 1881) announced that the cause of death was hepatic failure. Claude Bernard (1877) had given thought to this event and suggested that death resulted from effective hypovolaemia after trapping blood in the portal bed. Others (e.g. Tappeiner 1872) thought that more blood could be withdrawn from an animal which would survive, than could be contained in a distended portal bed, and that death occurred too quickly for either of the explanations to hold.

Nicolai von Eck opposed Schiff's reasoning. His dogs died in the same way as others until he thought of draining the ligated portal vein's blood into the vena cava. This he succeeded in doing in 1877 in a way ingenious for his time. He joined the two patent and unopened vessels side-to-side with a ring of closely placed sutures. A small opening, guarded by an untied suture allowed a pair of fine scissors to incise the two walls and open the veins to each other. The scissors were withdrawn and the remaining opening in the ring closed. The portal vein was then ligated on the hepatic side of the junction and the portal blood flowed into the vena cava.

The saga of his eight successfully operated dogs represents one of the great tragedies of all time in biology. Six died in the first fourteen post-operative days from infection, intestinal volvulus, thrombosis of the shunt and other complications of surgery. One dog remained alive and well two and a half months after the operation. One day the animal house attendant left the door open, and the dog

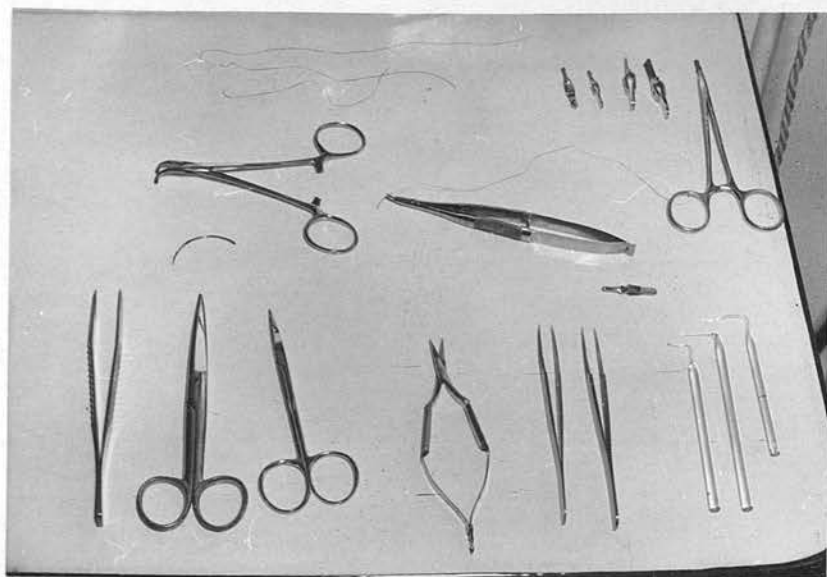


Fig. 9 Instruments used for porto-caval anastomosis  
in rats.

( $\frac{1}{4}$  scale).



vanished to freedom. Eck soon afterwards vanished into the Russian army never to be heard from again in scientific literature. This work and the suggestion that the operation might benefit some with certain types of liver disease enriched therapeutics, and has employed many experimentalists. (Pavlov 1893, Mann and Bollman 1926).

Whittaker performed porto-caval shunts in rats in 1946 by a method rather like that used by Eck. This method was described to me by Professor N.M. Dott who tried it many years ago, and who certainly used the same movements in creating his "aseptic" intestinal anastomoses (Dott and Fraser 1921). Whittaker placed a thread through the walls of the approximated vessels in a "horse-shoe" fashion, and used this instead of Eck's scissors to open the anastomosis.

A direct suturing method for end-to-side anastomosis, such as is now used in man was first performed by S.H. Lee (Lee and Fisher 1961). They claimed an operative mortality of under 5% in over 100 operations.

The method used in this study is similar to that of Lee. The method is quick, can be done single-handedly and gives total portal venous diversion. Lee ligates the right gastric (coronary) vein, a step which probably encourages re-establishment of venous drainage to the liver by opening lesser omental vessels to the porta hepatis.

An operative mortality of 10 - 15% was achieved, not quite as good as Lees' 5%.

#### Apparatus and Materials

Ether was the anaesthetic agent.

Special surgical instruments used were small bulldog clamps, a fine curved haemostat and ophthalmic needle holders (Fig.9.).

The suture material was 6 : 0's or 7 : 0's siliconised braided silk. The animals were of a Wistar Strain of rats bred in our department's animal house. Rats of between 140 and 270 grams body weight were used.

#### Anaesthesia:-

Anaesthesia was induced in a large glass jar - 3.5 cu.ft. into which a small piece of cotton wool soaked in ether was placed. Experience provided the knowledge of how much ether to give. In very cold weather smoother induction was facilitated by warming the jar; less pharyngeal secretion occurred then. Too high a concentration of ether also caused hypersecretion, as did a bottle of old ether in which toxic peroxides had presumably formed (Micks).

After induction, satisfactory regulation was achieved with open ether from cotton wool in a small jar. This was placed over the muzzle as required. Depth could be gauged by the characteristics of respiration, and by the tonus of the tail muscles. The dependent tail optimally hangs out at an angle of approximately  $45^{\circ}$ ; greater depth was indicated by approximation to the vertical.

#### Incision and Exposure:-

A mid line incision was made from the xiphi-sternal joint to the junction of the lower and middle thirds of the linea alba. The abdominal wall was held open by two sutures through the muscle and tied to staples stuck in the cork operating board.

The colon, small intestine and pylorus were packed to the left and caudally. This exposed the portal vein with the bile duct and hepatic artery, and, on their right the inferior vena cava.



Fig. 10 Porto-caval Anastomosis.  
The posterior wall is complete.  
The bulldog clamp is holding the  
portal vein. The mouths of the  
caval and portal vein openings  
are held open by stay sutures.

(X4)

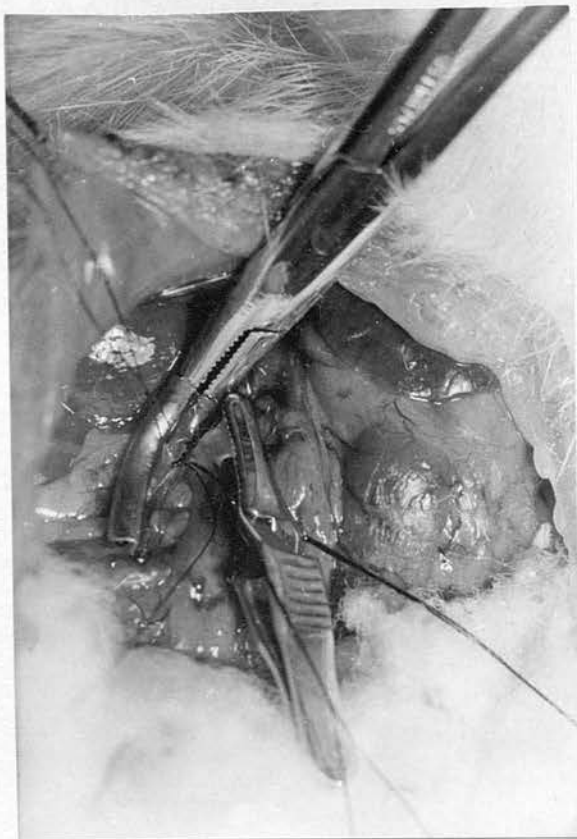
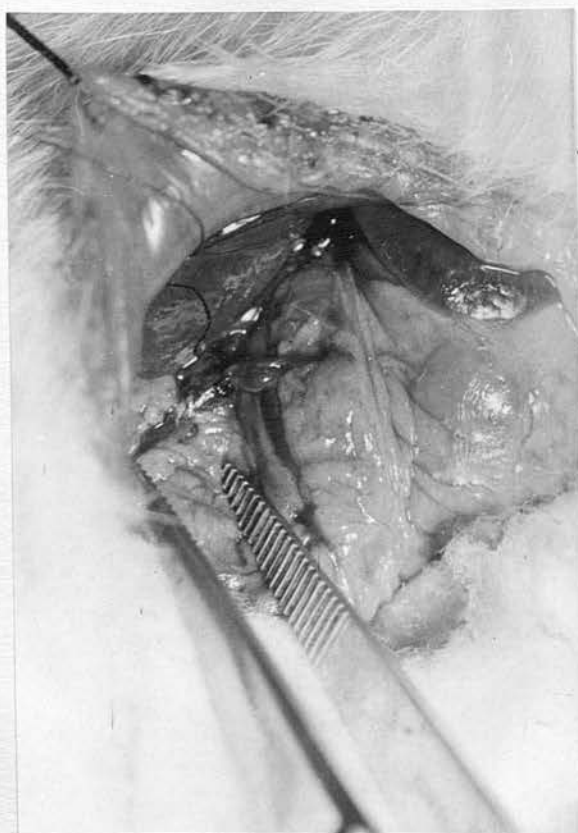


Fig. 11 Completed Anastomosis.  
The portal vein has a sharp bend  
in this picture. The right gastric  
joins the portal vein near the suture  
line.

(X 4).



The right lobe of the liver was lifted off the vena cava and fine bands of tissue divided. The vena cava was then cleared of serosa and fat from the liver to below the right renal vein. Dissection was also carried circumferentially around the vena cava to free it as much as possible. The portal vein was separated from the other structures in the free edge of the gastro-hepatic omentum by breaking through the omentum and gently easing them apart with glass dissectors. Great care was exercised to keep the right gastric vein intact.

#### Preparation for Anastomosis:-

When the portal vein was freed as far as the porta, a ligature was placed around it and half-tied in a loop so as to retain position but not to occlude the vein. This was then ready for ligation at a later stage.

At the point where the anastomosis was to be made, and the portal vein divided, two stay sutures were inserted in the wall of the vein and held by clamps some distance from the vein. Slight bleeding usually occurred on inserting these stays, but with slight pressure from pledgets of cotton, the bleeding points rapidly closed.

#### Anastomosis:-

Curved mosquito forceps, ground down and shod with polythene were placed on the vena cava, to catch up a bleb on its anterior wall. The handles of the clamp were suspended out of the way, on threads. The bleb in the vena cava was stretched to a flat diamond by laterally placed stay sutures and an opening made longitudinally between these.

The portal vein was then clamped with a small bulldog clamp. This clamp was positioned to give the greatest possible free length of vein, after the ligature in the porta

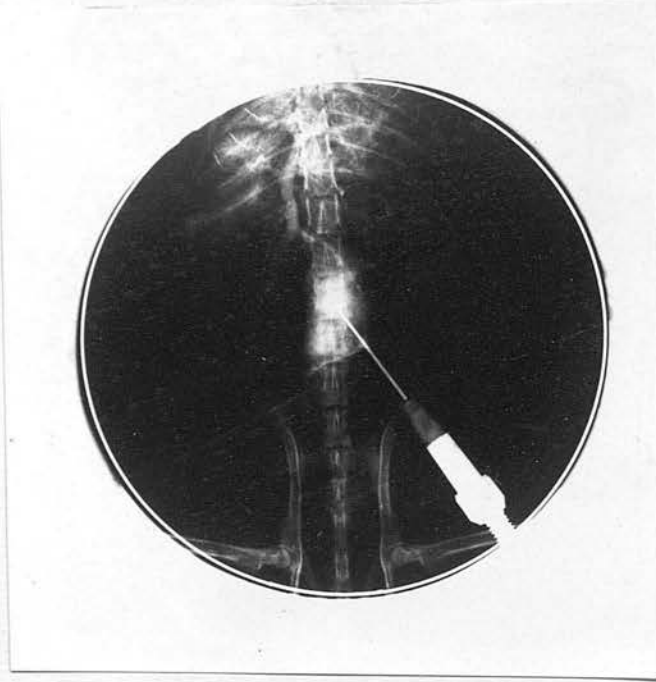


Fig. 12 Splenopottogram in a normal rat. The needle is in the spleen, in which the contrast medium has diffused. The splenic vein is outlined running obliquely to meet the portal vein, which runs vertically to its division. Intrahepatic branches of the portal vein can be seen at the top of the picture.

(life size).

had been tied and the vein divided. The open mouth was then swung over to the aperture in the vena cava.

A continuous 6 : 0 or 7 : 0 suture was used on an atraumatic needle. The first stitch at the rostral end, when knotted, held the two vessels together. The posterior wall was completed, and a locking stitch inserted to prevent "purse-stringing" (Fig.10) The anterior wall was then completed as rapidly as possible. The clamps were removed as soon as the last tie was made (Fig.11). The visceral circulation re-established itself rapidly. The duration of clamping of the portal vein was usually under 12 minutes.

#### Post-operative Care:-

Usually none was required.

When the operating time was about 20 minutes the animal was walking gingerly within 10 minutes of the operation's end. Longer operations or deeper anaesthesia were associated with longer post-operative recovery periods. Such animals were nursed in warm boxes until they were lively enough to be put back in their cages.

All animals were housed in individual cages, and fed on an unlimited diet of standard rat cake and water. No pre- or post-operative restrictions were made.

#### Patency of Anastomosis:-

Patency was maintained in all animals surviving over six hours. This was tested by angiography in life (Figs. 12-14) or by dissection and perfusion at autopsy. The radiographs were kindly taken by Dr. Bruce Young. Still films were taken with a tube-film distance of 20 inches, using broad focus at 36 kv 400 ma for 0.6 secs. The film used was Kodak-rex, and Angioconray 60 was the contrast medium employed.



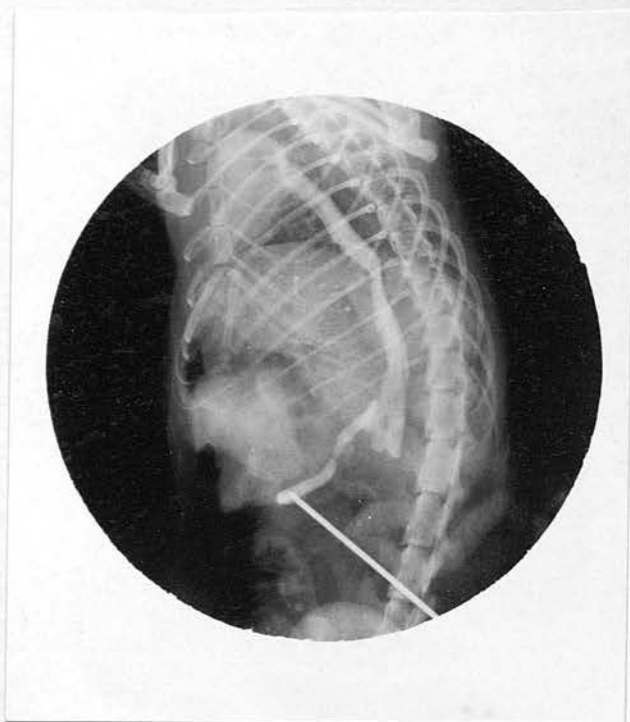


Fig. 13 Mesenteric portogram in a rat with porto-caudal anastomosis. The needle is in a branch of the superior mesenteric vein. The contrast medium delineates the superior mesenteric vein, the portal vein, the anastomosis, vena cava, and heart.

(life size).

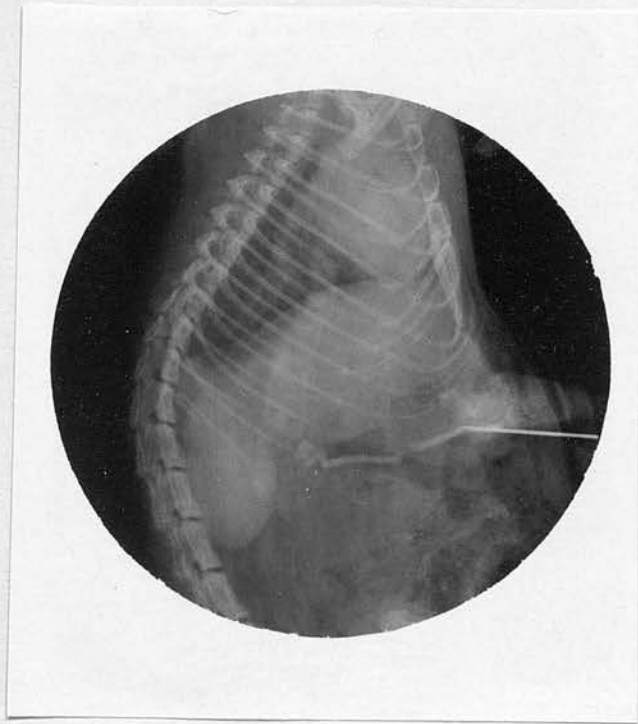


Fig. 14 Splenoportogram in a rat with porto-caval anastomosis. The needle is in the spleen. Contrast medium is in the spleen, splenic vein, portal vein, and, more faintly, in the vena cava and heart.

(life size).



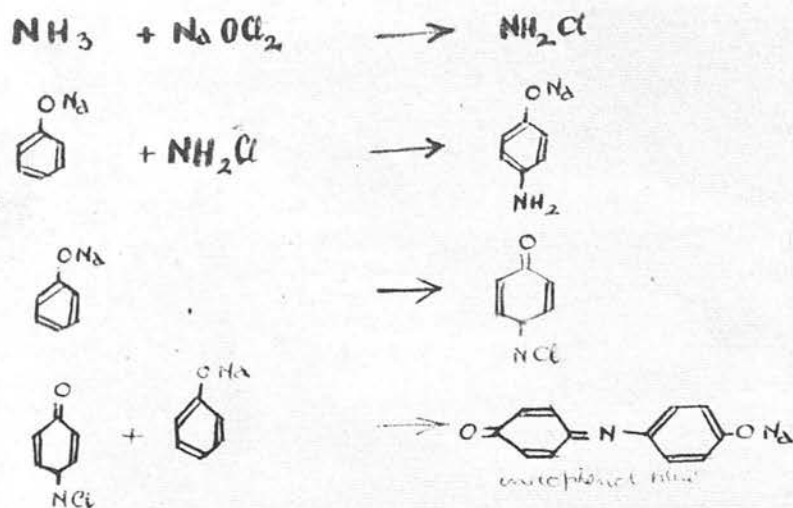
Sham Operation:-

The sham operation comprised exposure, dissection and clamping the vessels in the same way and for the same times as in the full operation.

Times for all stages of the operation were noted and recorded at each operation.

AMMONIA

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FENTON'S METHOD FOR AMMONIA - COLOURIMETRIC REACTION.

Fig. 15.

## AMMONIA

### Available Methods and Reasons for Selection:-

E.J.Conway's classic microdiffusion techniques made possible the detection of quantities of ammonia as small as are present in human blood. The essentials of the method are that in a small, confined space a volatile substance will diffuse from the solution from which it is evolved to another where it is trapped.

Ammonia is liberated from freshly drawn blood by the displacing action of a strong alkali. This property of alkalies is complicated in blood by the breakdown of amines and the artificial production of ammonia. The diffusion of the evolved ammonia to the acid trap takes 20 minutes. At room temperature decomposition of ammonia yielding substances occurs and adds a second source of error. Both can be allowed for by calculation.

Apart from these sources of potential error, the problems of time and resources make the Conway method a forbidding undertaking for the solitary worker. A large stock of non-utilitarian glassware must be maintained in a state of perfect chemical cleanliness. A special room is usually required where no danger of contamination with acid or alkaline vapours exists.

Conway's method and its many modifications, on which the work on ammonia metabolism has been based, has been superseded by a chemically "cleaner" method, described by Fenton (1963).

Fenton's method comprises the adsorption of free ammonium ions from freshly drawn, separated and chilled plasma on to a cooled, strong cation exchange resin. The ammonium ions are subsequently displaced by concentrated sodium chloride and trapped by a specific reaction with alkaline sodium hypochlorite and phenol. The resulting blue indophenol (Fig.15). is directly proportional in intensity to the concentration of ammonium ions in plasma.



|                     |     |                |                        |                  |   |
|---------------------|-----|----------------|------------------------|------------------|---|
| <u>Normal Rats:</u> | 32  | $\mu\text{gm}$ | $\text{NH}_4\text{-N}$ | /100 ml. plasma. |   |
|                     | 35  | "              | "                      | "                | ※ |
|                     | 62  | "              | "                      | "                | ※ |
|                     | 72  | "              | "                      | "                |   |
|                     | 142 | "              | "                      | "                | ※ |
|                     | 170 | "              | "                      | "                |   |

Mean 85.5  $\mu\text{gm}$   $\text{NH}_4\text{-N}$ /100 ml. plasma

※ indicates sham-operated animal.

Rats with Porto-caval Anastomosis:

|  |         |     |                |                        |                  |
|--|---------|-----|----------------|------------------------|------------------|
|  | 30 days | 370 | $\mu\text{gm}$ | $\text{NH}_4\text{-N}$ | /100 ml. plasma. |
|  | 63      | "   | 500            | "                      | "                |
|  | 103     | "   | 400            | "                      | "                |
|  | 218     | "   | 340            | "                      | "                |

Plasma Ammonia-nitrogen Values for Normal, Sham-operated and Rats with Porto-caval Anastomosis.

Fenton's method as described in Clin.Chem.Acta (1963) was followed.

#### METHOD

Four rats with porto-caval anastomoses patent for more than 21 days, three sham-operated rats and three normal rats were used. The necessity for duplicate readings required exsanguination of the animals. More than 8 ml. blood was required for two estimations.

The rats were anaesthetised with ether and the chests opened. Blood from the left ventricle was drawn into a chilled, chemically clean syringe containing a few drops of heparin. When approximately 10 ml. blood had been drawn it was transferred to chilled centrifuge tubes and spun at 0°C. for 5 minutes to separate the plasma. 2 ml. of plasma could then be drawn from each tube, applied to the columns and run through in 15 minutes.

After washing the active columns with ammonia-free distilled water, the ammonia was displaced by 4 Molar sodium chloride into a tube containing ammonia-free Aluminium sulphate.

The elution was completed with a final wash with water. The ammonium is then prepared for reaction with sodium hypochlorite and alkaline phenol.

Calibration of the colour reaction was by prepared dilutions of ammonium sulphate. The accuracy of the method is impressive.

#### RESULTS

These are shown in Fig. 16.

The mean value for the normal and sham-operated group was 85.5  $\mu\text{g}$   $\text{NH}_4\text{-N}/100$  ml. plasma, with 32 and 170 as the low and high figures.

The rats with porto-caval anastomosis had plasma ammonium levels of four times the normal group. The mean value was 400  $\mu\text{g}/100$  mls plasma. The level bore no relationship to time after anastomosis, as far as can be

assessed from such a small series. It was not possible to estimate the ammonium levels of stuporose rats which will be described later. These were probably in extremis and not truly encephalopathic. It was impossible to draw sufficient blood from them for estimation.

#### DISCUSSION

It is difficult to compare the values given by Conway's and Fenton's methods, because the former is from blood, and the latter plasma. It would seem from the normal values of each for humans that the following must be equivalent:-  
1 - 2  $\mu\text{gm}/\text{ml}$ . blood or 100 - 200  $\mu\text{gm NH}_4\text{-N}/100 \text{ ml. blood}$  by Conway (Sherlock et al)

is equivalent to 3 - 27.5  $\mu\text{gm NH}_4\text{-N}/100 \text{ ml. plasma}$  by Fenton (1963).

In 17 normal rats studied by Lee and Fisher (1961) the levels were

$220 \pm 60 \mu\text{gm}/100 \text{ ml. blood}$ .

and after shunting  $610 \pm 160 \mu\text{gm}/100 \text{ ml. blood}$ .

Allowing for the differences of normal values for the two methods, and correcting for plasma as against blood, the rats in my series seem to have a lower normal values and slightly higher post-shunting values.

None of the rats in this series had ammonium measured during anything resembling stupor or coma. None of Lee's animals showed such phenomena. Levels for humans in coma are given as over 800  $\mu\text{gm}/100 \text{ ml. plasma}$  by Fenton, and, by Conway's method, levels of less than 100  $\mu\text{gm}/100 \text{ ml. blood}$  to 600  $\mu\text{gm}/100 \text{ ml. blood}$  were found by Sherlock (1958).

Less abnormal levels are reported by both Fenton and

Sherlock in people in pre-coma, and up to 600 ugm/100 ml. plasma or 50 - 450 ugm/100 ml. blood respectively by the same authors. The rats done here and in Pittsburg all seem to enter the range considered probably toxic for humans, but show no clinical neurological or behavioural abnormalities.

This could cast doubts on the validity of the ammonia intoxication theory for acute hepatic encephalopathy, but rats have higher normal values than humans, more than twice as high. This may result from the habit of coprophagia in rats, with consequent recirculation of nitrogenous substances. The rat may be more tolerant of ammonium than is man, for this reason and probably very much higher levels would be required before a rat would show signs of toxicity.

#### SUMMARY

Normal rats have circulating ammonia nitrogen levels of  
 85.5 ugm/100 ml. plasma (Fenton's method)  
 or 220 ugm/100 ml. blood (Conway's method).  
 Rats with porto-caval anastomosis have levels of  
 400 ugm/100 ml. plasma  
 or 610 ugm/100 ml. blood.

No definite signs of encephalopathy have been observed in rats.



CHANGES IN BODY WEIGHT AND POST-OPERATIVE  
SURVIVAL INCLUDING GASTRIC EROSIONS

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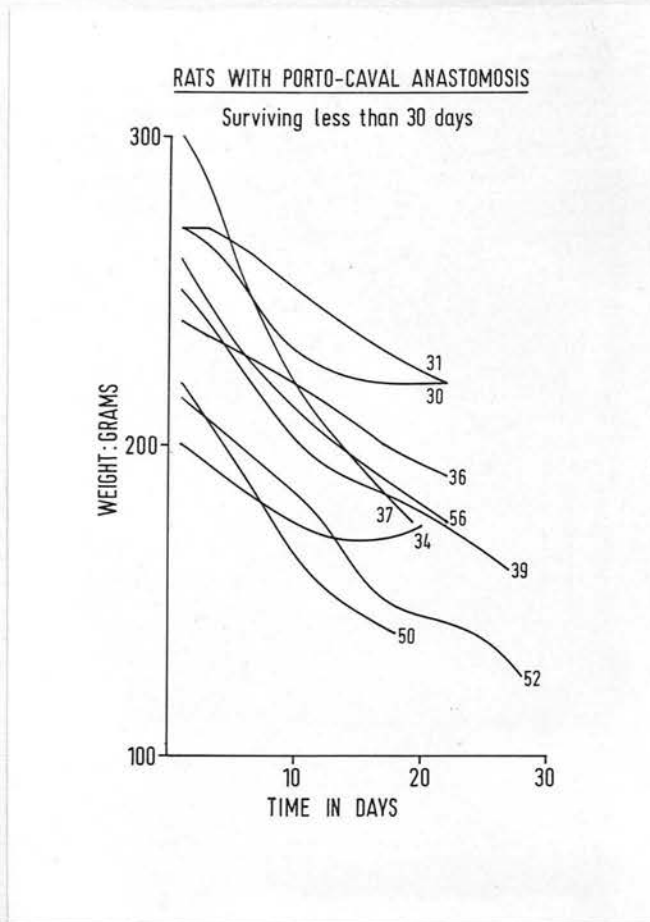


Fig. 17 depicts the body weight changes with time after porto-caval anastomosis. These rats either died or were killed before 30 days. The number at the end of each trace is the individual's serial number.

### CHANGES IN BODY WEIGHT AND POST-OPERATIVE SURVIVAL

35 rats surviving the operation were used for this part of the study. Measurements of these have provided a pattern which has been followed by rats studied subsequently and which show a general agreement with the observations of Lee and Fisher (1961) and Bismuth (1963).

Rats of between 140 and 300 G body weight were used. No difference in behaviour of the sexes was noticed.

In the first three weeks after operation there was a dramatic fall of body weight, as much as 50%, with an average loss of 40% of the original weight (Fig.17). This occurred in spite of apparently undiminished appetite and unlimited supplies of food. During this period there was a heavy mortality.

8 of the 35 died, and of these 5 died between 4 and 19 days. The animals at this time had severe depletion of body fat. They were reduced to "skin and bone" but seemed normally active. The only possible sign of encephalopathy was, as shall be described more fully later, a terminal episode of stupor or coma in some.

Rats surviving the first three weeks passed through a phase of relative stability of weight for a further three weeks (Fig.18). 7 of the 35 died in this period. From six weeks onwards there was a tendency to regain weight, but only a few returned to their pre-operative weight, and fewer surpassed it (Fig.19).

The original growth curve was never resumed.

The figures are contrasted with the short post-operative weight fall in sham-operated rats. This drop of under 5% occurred in the first post-operative week and these rats had returned to their normal growth curves within two weeks.

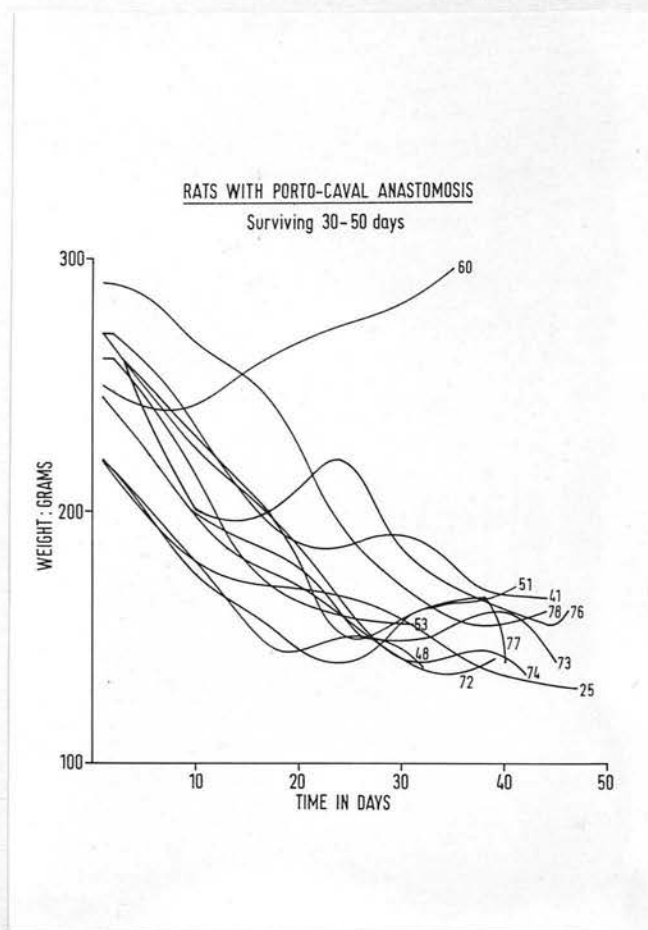


Fig. 18 depicts the body weight changes with time after porto-caval anastomosis in animals which were killed or died between 30 and 50 days after operation. The number at the end of each trace is the individual's serial number. Rat 60 is a sham-operated animal.



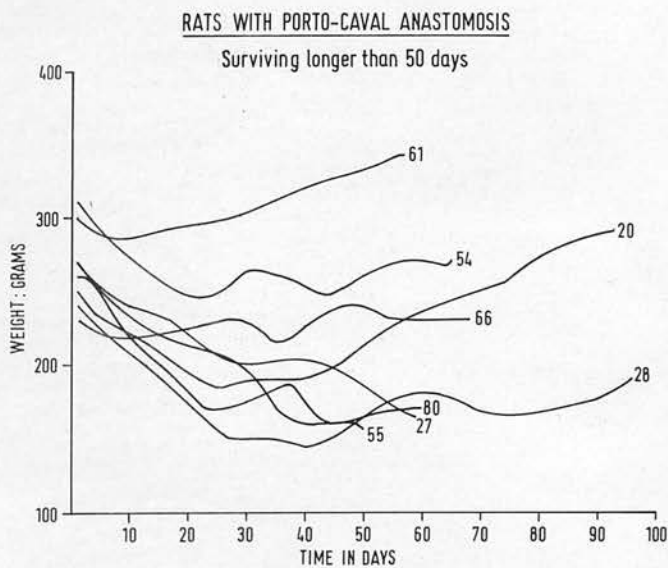


Fig. 19 depicts the body weight changes with time after porto-caaval anastomosis in animals surviving the operation by 50 days or more. The number at the end of each trace is the individual's serial number. 61 is a sham-operated rat.

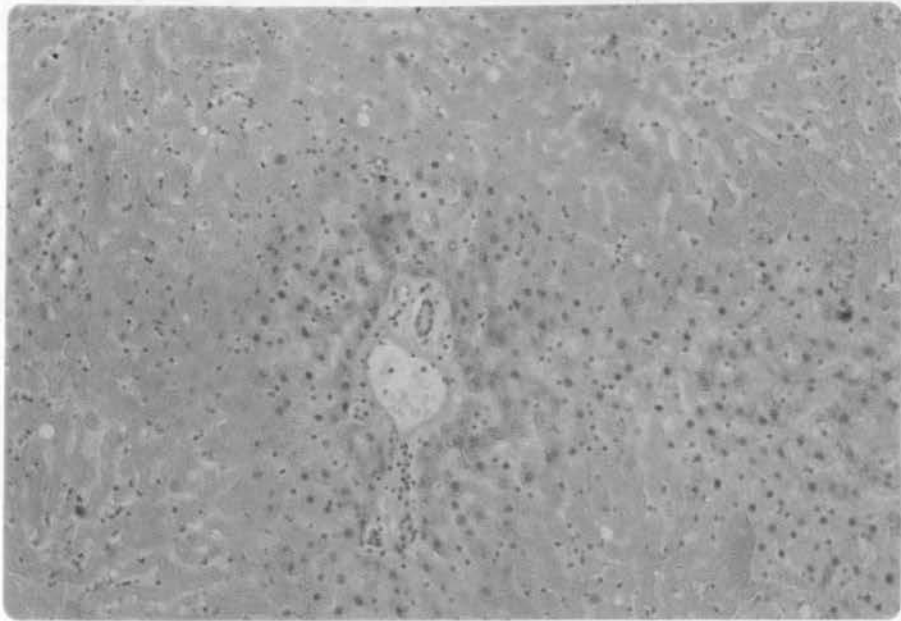


Fig. 20 Haematoxylin and eosin stained section of the liver of a rat which died 36 hours after operation. The anastomosis was occluded with thrombus and the gut was deeply congested. The hepatic artery was not damaged during the operation. The picture is one of almost complete necrosis, only a few parenchymal cells surviving around the portal areas.

## CAUSES OF DEATH

### Patency of Anastomosis

If the anastomosis remained patent past the fourth post-operative day it remained patent indefinitely. A few rats are included in Fig.7, which died during the first four days with thrombosis of the anastomosis, and severe venous congestion of the gut. In nearly all of these a picture of complete devascularisation of the livers is seen on histological examination (Fig.20). This occurred in spite of the fact that particular attention was paid to the separation of the hepatic artery and bile duct from the portal vein during preparation for anastomosis, and checking that the artery had not been caught up in the ligature for the portal vein or its clamp.

That the anastomoses remained patent after four days was demonstrated by angiography in life or by careful dissection and perfusion in cadavers.

## GASTRO-INTESTINAL BLEEDING AND GASTRIC EROSIONS

### Introduction

The association of gastric erosions and liver disease has been known for at least fifty years (Fenwick and Fenwick, 1910). That there is increased gastric acid secretion after experimental porto-systemic anastomosis has been shown from innervated gastric pouches - Lebedinskaja (1933) -, intact stomachs - Gregory (1958) - and denervated pouches - Dubuque (1957). There is confirmation of the increased gastric acid secretion in this situation in man - McDermott (1965) - and in rats - Day et al (1963). Irvine (1959) demonstrated elevation of circulating histamine concentrations following shunting procedures, and Day et al (1963) demonstrated similar elevation in rats. The incidence of peptic ulceration following shunting in man is estimated about 15% of cases - Wilkinson (1965).



Fig. 21 Everted stomachs of 3 rats with bleeding from gastric mucosal erosions. The erosions are dark brown spots on, and adjacent to, the rugae. No lesions of the squamous, ruminal stomach were seen.



## RESULTS

Of the 26 female rats with porto-caval anastomosis, 16 had gastric erosions with haemorrhage. The remaining 10 had neither lesions nor blood in the gut. Two of the 10 erosion-free animals had been noted to have melaena stools several weeks before death, but no source of haemorrhage was found at autopsy.

Of the 12 males, 9 had erosions.

There was no relationship between age and the development of lesions. Fig. 22 indicates how the lesions presented in the males and females, in those dying and in those killed. It shows that the post-operative period in which ulcers were most frequently found was between 19 and 45 days, but they were seen as late as 96 days.

The presence of altered blood in the intestinal contents was confirmed by "Haematest" (Ames Co.Ltd.).

No haemorrhage was observed, nor ulcers seen in the gastrointestinal tracts of normal or sham-operated rats.

No thrombosis of anastomosis was found in rats with erosions.

## GASTRIC PATHOLOGY

Acute erosions were found in the glandular part of the stomach. No abnormalities were noticed in the squamous-lined, ruminal stomach, oesophagus or duodenum. The number of lesions varied from two to countless and in size from punctate to 1.5 mm. diameter. They were found on the crests of rugae or, when large, encroached on adjacent areas (Fig. 21).

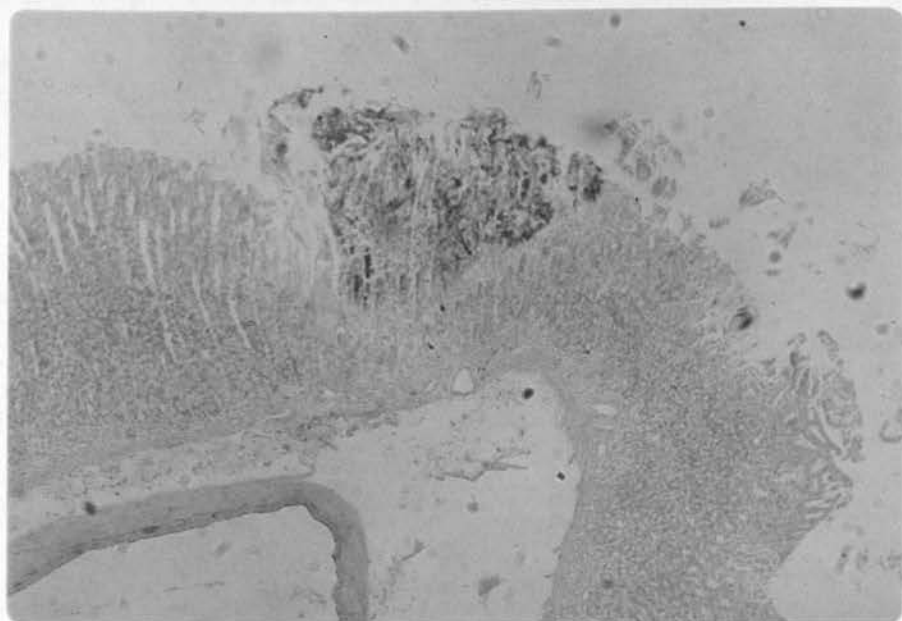
The quantity of blood in the alimentary tract, either as fresh "coffee grounds" in the stomach, blue discolouration of the small intestine or as melaena in the colon, often suggested that bleeding had been continuing for some days. In others, although the quantity of blood freshly released seemed small, melaena stools were evidence that bleeding had been continuing for some weeks. These were severely anaemic

|                  | Sex | Mode of demise | No. of animals | Time (days) after operation | Distribution of time of death |    |    |    |    |    |    |    |    |     |     |  |  |  |
|------------------|-----|----------------|----------------|-----------------------------|-------------------------------|----|----|----|----|----|----|----|----|-----|-----|--|--|--|
|                  |     |                |                |                             | 10                            | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 |  |  |  |
| with erosions    | F   | died           | 13             | 1 - 62                      |                               |    |    |    |    |    |    |    |    |     |     |  |  |  |
|                  | F   | sacrificed     | 3              | 19 - 96                     |                               |    |    |    |    |    |    |    |    |     |     |  |  |  |
|                  | M   | died           | 7              | 21 - 50                     |                               |    |    |    |    |    |    |    |    |     |     |  |  |  |
|                  | M   | sacrificed     | 2              | 41 - 48                     |                               |    |    |    |    |    |    |    |    |     |     |  |  |  |
| without erosions | F   | died           | 5              | 4 - 92                      |                               |    |    |    |    |    |    |    |    |     |     |  |  |  |
|                  | F   | sacrificed     | 5              | 21 - 103                    |                               |    |    |    |    |    |    |    |    |     |     |  |  |  |
|                  | M   | died           | 2              | 4                           |                               |    |    |    |    |    |    |    |    |     |     |  |  |  |
|                  | M   | sacrificed     | 1              | 63                          |                               |    |    |    |    |    |    |    |    |     |     |  |  |  |

Distribution of gastric erosions among rats with porto-caval anastomosis by sex, mode of death, and time after operation.

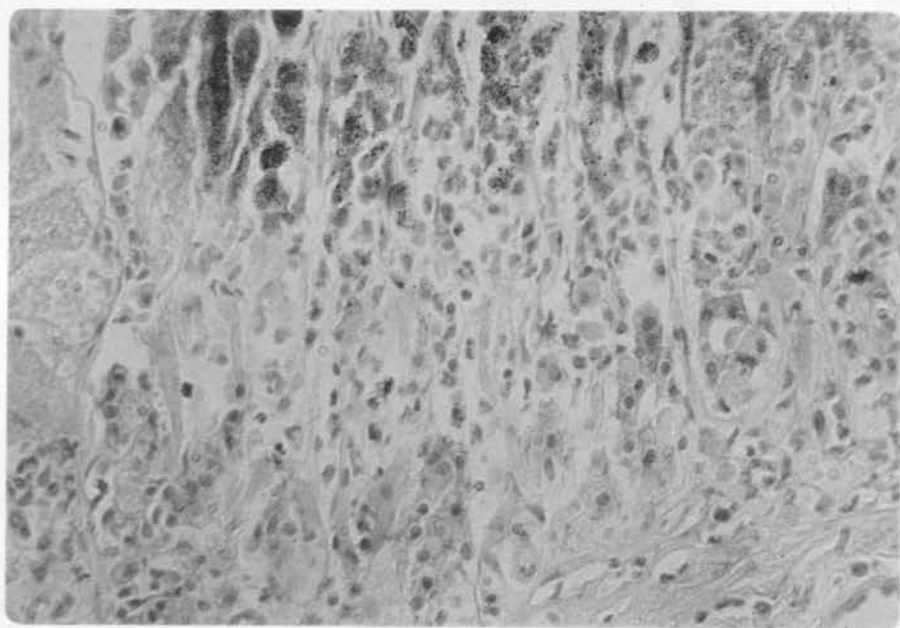
Fig. 22.

Fig. 23



The erosions are small, punched-out areas, well-demarcated from adjacent, healthy mucosa, but with small numbers of polymorphonuclear leukocytes in the margin between healthy and necrotic tissue.

Fig. 24



and the further small haemorrhage may have been sufficient to cause death. Erosions were present in animals dying, as well as in those killed by perfusion of fixatives.

#### MICROSCOPIC FEATURES

The lesions satisfy the definition of mucosal erosions, viz., a breach in gastric mucosa which does not penetrate muscularis mucosae. Some very superficial erosions resemble the "Leistenspitzenerosionen" described in Henke and Lubarsch (1928).

Diameters of 0.5 - 2 mm. were the rule with sharply defined margins giving a "punched out" appearance. The immediately surrounding mucosal cells appeared healthy. The craters contained bile-stained debris. There were no thrombosed or occluded vessels in the vicinity. Inflammatory reaction was minimal, a few polymorphs being found in the immediately adjacent mucosa or in the tissues of the base.

No chronic lesions were found.

#### ASSOCIATION OF THE EROSIONS WITH OTHER FINDINGS

Normal control, and sham-operated rats grew and thrived normally under the conditions of the experiment. None of these rats had erosions.

Two animals with erosions had suppurative bronchopneumonia with numerous large Gram-negative bacilli in the sections of the lung. One had suppurative otitis media and acute, purulent leptomeningitis.

Body weight losses of up to 40% occurred in the operated rats during the first 20 days. Thereafter, following a period of relative stability of 10 - 30 days at the reduced weight, body weight tended to climb again until some of those surviving sufficiently long recovered the loss



or surpassed their original weight. The erosions were more common during the period of stability but were not confined to this period.

The weight changes occurred independently of food intake. There was no diminution of appetite and food intake of the balanced rate cake and water was not restricted. The situation was not considered analagous to the starvation ulceration reported by some authors (vide infra).

#### ADRENAL AND PITUITARY GLANDS

There was no detectable histological difference between the pituitary or adrenal cortical cells of those with or without gastric lesions, or of normal or sham-operated controls. No evidence for hyperplasia of cortical cells could be adduced from thymidine autoradiography. Acute ulcers can be produced in the glandular parts of the rat's stomach by starvation (Wiemeggers, 1964; Baghavan, 1965), isolation and restraint (1948). Ulcers of the ruminal (squamous) stomach were reported by Buchner (1928). These ulcers rapidly followed massive histamine injection in many of their starved, isolated, restrained rats.

In this series neither restraint nor food deprivation occurred. Elevated circulating histamine levels may have increased acid secretion, but because of the relative resistance of the normal rat's gastric mucosa to histamine ulceration, it is suggested that this mechanism may at best be additive in causing ulceration.

There was no histological evidence of adrenal cortical hyperfunction to implicate an endocrine effect of the mucosa.

## DISCUSSION

The pattern of weight change in these rats following porto-caval anastomosis concurs with other studies of a comparable number of animals (Bismuth, 1963). There was no substantial difference in the operative technique nor maintenance of the animals. Gastro-duodenal ulceration did not occur in Bismuth's series. Day et al. (1963) in a study of gastric secretion following shunts in five rats found multiple acute erosions in one and they infer that this might have followed trauma during gastric aspiration. They found acid secretion increased and blood, liver and stomach wall histamine concentrations elevated.

Rats are said to destroy histamine efficiently at the gastric mucosa because of the high histaminase activity there. This may be a reason why histamine ulceration is more difficult to produce in rats than in other mammals (Wynn-Williams, 1959). Acute ulcers can be produced in the glandular parts of the rat's stomach by starvation (Niemeggers, 1964; Raghavan, 1965), isolation and restraint <sup>Gruddjan</sup> (1948). Ulcers of the rumenal (squamous) stomach were reported by Buchner (1928). These ulcers rapidly followed massive histamine injection in many of their starved, isolated, restrained rats. <sup>had had bleeding</sup>

In this series neither restraint nor food deprivation occurred. Elevated circulating histamine levels may have increased acid secretion, but because of the relative resistance of the normal rat's gastric mucosa to histamine ulceration, it is suggested that this mechanism may at best be additive in causing ulceration.

There was no histological evidence of adrenal cortical hyperfunction to implicate an endocrine effect of the mucosa.

Although there was a slight increase in the weight of these glands (Fig. 73. & 74) Crean (1965) has shown a reduction of mucosal cell mass after steroid hormone and ACTH administration.

The gastric mucosa in the non-eroded areas appeared normal at simple non-quantitative microscopic examination. In the surrounds of the lesions minimal polymorph reaction was found. This paucity of inflammatory cell infiltration seems to be a characteristic of the experimentally induced erosions in rats, and Figs. 23. and 24. compare in this respect with the illustrations of Basu Mallik of pilocarpine induced lesions.

Erosions were found in animals dying and in those killed by perfusion of fixatives. This indicates that the lesions were probably not agonal phenomena.

The finding of bleeding from the gastro-intestinal tracts in two animals which subsequently survived for weeks and had no lesions at post mortem may signify that these erosions healed without scarring.

#### SUMMARY

Of the 38 rats with end-to-side porto-caval anastomosis, 25 developed acute gastric erosions and two had had bleeding from the gastro-intestinal tract but no responsible lesion was discovered at autopsy.

THE LIVER FOLLOWING ANASTOMOSIS

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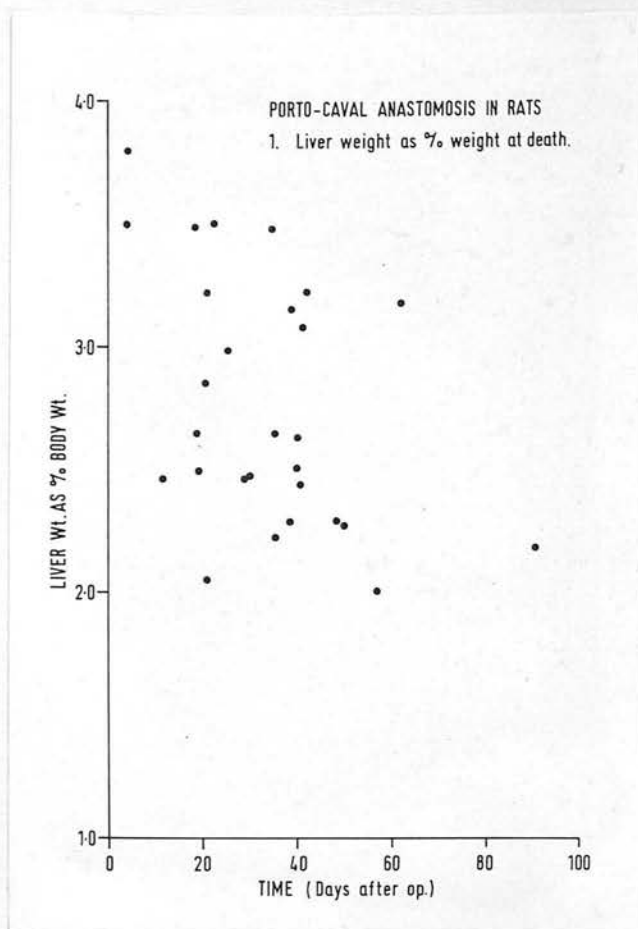


Fig. 25 Liver weight, as a percentage of body weight, falls after operation. The percentage of body weight occupied by the liver in a normal rat is about 4.5%. Animals followed to over 200 days had livers between 2.0 and 2.5% of body weight. The overall picture was a severe fall of liver mass in 20-40 days, without subsequent recovery.

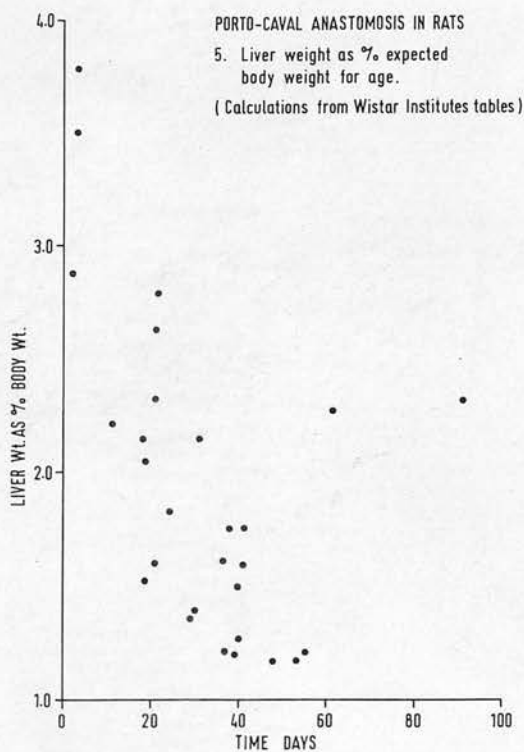


Fig. 26. As shown in fig's. 17, 18, 19, there are profound changes in body weight which complicate interpretation of liver mass fluctuations. This figure shows the liver weight at death as a % of the expected body weight of an animal of the same age which had had no porto-caval shunt. This emphasizes the changes shown in fig. 25. The two "aberrant" points high on the right are of livers which had inter-lobular abscesses, and were heavier because of associated pus.

### THE LIVER FOLLOWING ANASTOMOSIS

The liver weights (as with other organ weights) have been compared with the large tables of figures for Wistar rats from the Wistar Institute (H.H. Donaldson, 1928). It was found that the organ weights of the Wistar Strain of our animal house agreed sufficiently with the Institute's series that it would probably be acceptable to use it as a basis for comparison. The growth curves were found to be closely aligned.

The liver weight noted at autopsy, and the following comparisons drawn: (i) liver weight as a % of body weight at death (Fig.25);

(ii) liver weight as a % of the "expected body weight" at death (Fig.26).

The "expected body weight" was the weight the animal should have reached had the operation, with its weight reduction, not occurred, i.e., body weight for age. This figure was thought desirable to try to eliminate the variation of body weight loss caused by the operation. The reduced liver mass might be related more to this than the reduced body weight.

(iii) liver weight as % of "expected liver weight" at death (Fig.27).

The "expected liver weight" is the approximate weight the liver should have been for the body weight at death. The liver weight bears a remarkably constant proportion to body weight. This figure should show whether the weight of the liver is in normal proportion to the reduced body weight.

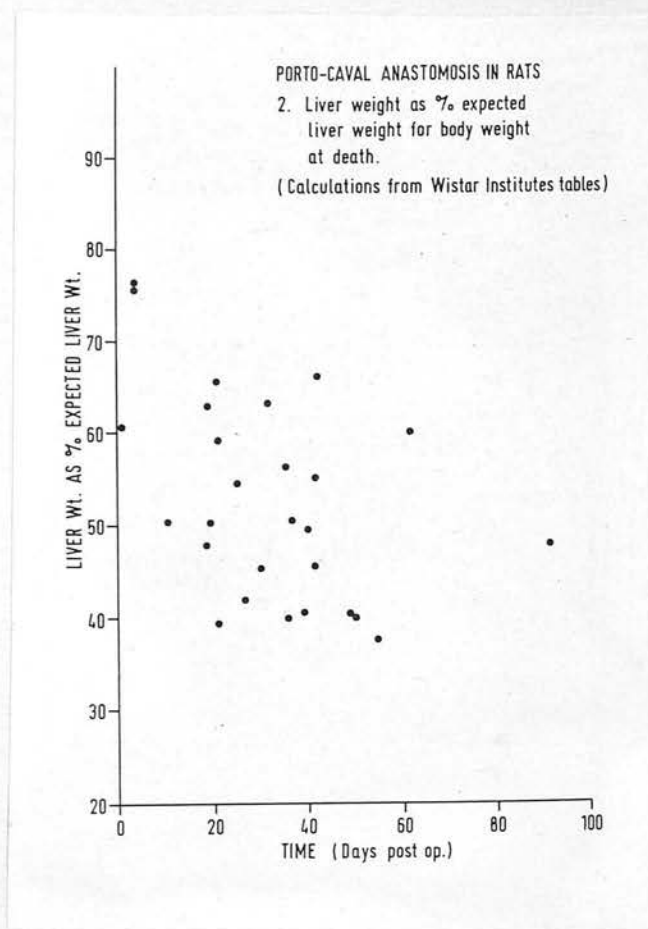


Fig. 27. This figure relates the liver weight at death to the liver weight expected in an animal of the same body weight. It shows that the fall in liver mass is approximately 60%. This low value was maintained to over 200 days survival.



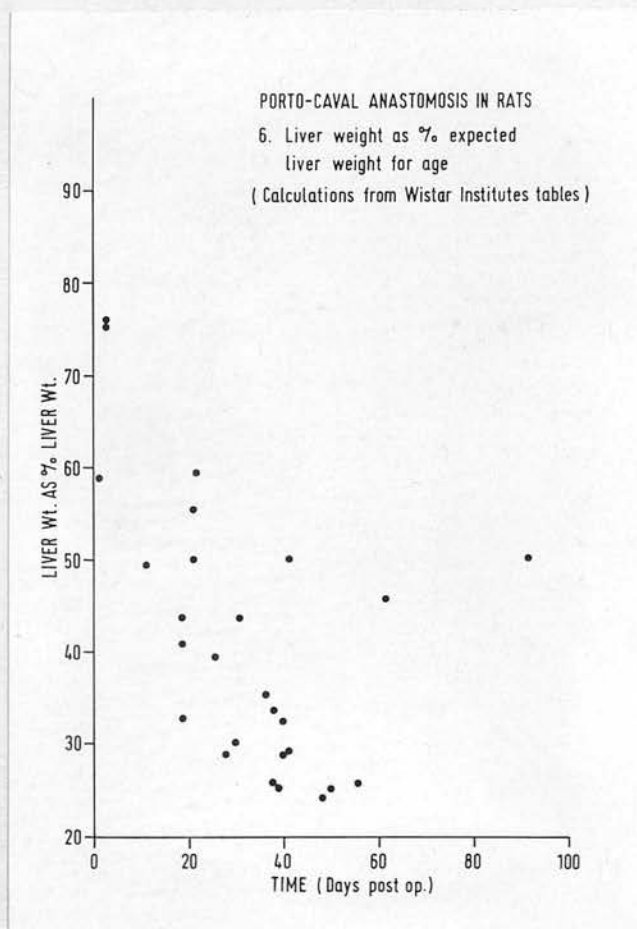


Fig. 28 The liver weight is related to the weight expected in an animal of the same age, but without porto-caval anastomosis. The two points high on the right are from the rats mentioned in fig. 26.

(iv) liver weight as a % of "expected liver weight for expected body weight" at death (Fig.28).

The "expected liver weight for expected body weight" at death is an attempt to relate the weight at death to the weight the liver might have been for that age of animal had operation not intervened.

Of the four simple calculations, the first is the most valid, because it does not draw on any outside figures for comparison.

The normal rat's liver is approximately 4.5% of body weight.

In all the rats the reduction of liver mass is seen to be in excess of what might be considered proportionate to the body weight fall. The figures at death are all below 3%. From these calculations it is seen that the liver is about 40% of its expected size.

This level of approximately 40% of the expected size is maintained throughout the subsequent course of the animal's life. Rats dying at over 200 days, having recovered body weight, still have small livers, but the proportion to body weight is apparently fixed at 40%.

One rat which survived shunting and partial hepatectomy at one operation was killed at 35 days. Its liver was approximately 45% of the expected weight for that size of animal.

#### OBSERVATIONS ON THE POWER OF THE LIVER TO REGENERATE AFTER PORTO-CAVAL ANASTOMOSIS

The liver weight is considered to be reduced to 40% of its expected weight after anastomosis. Three weeks after removal of

50% of the liver substance at the time of operation, the liver mass was 45% of the expected weight.

60% partial hepatectomy, removing the major parts of both right anterior and middle lobes (6.2 G), was performed in one rat three weeks after porto-caval shunt. Three weeks after partial hepatectomy the liver mass was 37% of that expected for the animal's body weight. The conclusion drawn from this is that, provided the rat's liver had followed the pattern of all the others up till three weeks, partial hepatectomy was followed by regeneration of the liver to the new low weight. The power of the liver to regenerate was not impaired.

#### DISCUSSION:

The liver has been shown to have exceptional power of regeneration in all mammalian species.

The important problems in regeneration of the liver are, firstly the nature of the stimulus to activity, secondly, what stops it, and thirdly why the liver mass is so well controlled. In the normal rat, partial hepatectomy is followed by a burst of regenerative activity which, beginning within 12 - 24 hours takes a remnant of 50% to near the full hepatic complement in 7 days.

Mitotic activity is usually finished by ten days after 50% hepatectomy.

It was formerly thought that the liver's power to regenerate

was either abolished or severely compromised (Child et al. 1954) after porto-caval anastomosis. Direct portal venous perfusion of the liver was also thought to be essential to regeneration. This latter point was disproved by transposing the portal and vena caval flows, and showing that regeneration occurred in the liver perfused with caval blood (Child et al). The liver's arterial perfusion continues, and, all the substances carried by the portal vein should reach the liver by that route after the shunt, unless they are handled elsewhere.

Weinbren has shown that ligation of the portal branches to lobes of the liver does not impair regeneration in the segments only supplied by arteries.

It would seem important that the rat's liver should reach a new low size after shunting, and that this low mass is regained after partial hepatectomy in such animals. Lack of appreciation of the reduction of mass may have given rise to the belief that the regenerative ability is compromised. From the experiment quoted above it would appear that there is full ability to regenerate. This work is confirmatory of the Fishers' (1962).

#### HISTOLOGICAL EXAMINATION OF LIVERS

Of the 35, two have already been discussed, namely those dying inside 4 days with devascularised, infarcted livers. These showed a tendency to survival of the periportal parenchyma.

29 of the remaining 33 have livers which, without the evidence of



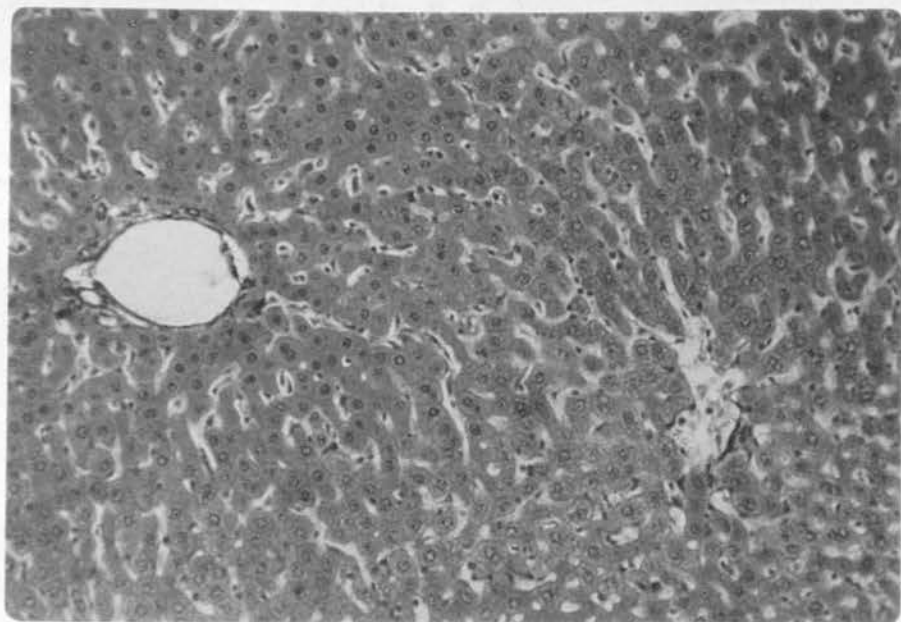


Fig. 29

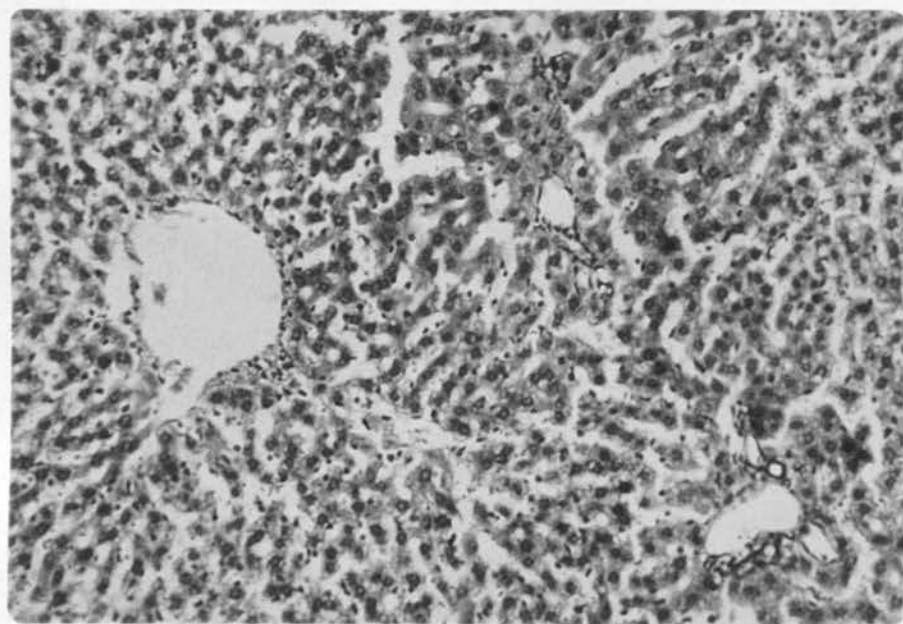


Fig 30

Figs. 29 and 30 are from Masson stained paraffin sections, both handled in the same way. 29 is of a sham-operated animal 21 days after operation. 30 is of a rat 21 days after porto-caecal anastomosis. Shrinkage of the cytoplasm and dilatation of the sinusoids is shown.

(both x 150)



Fig. 31 Gordon and Sweet's reticulin stain on the same liver as shown in fig. 30. There is collapse of reticulin, but the basic pattern of the tissue is not altered.

(x 300).

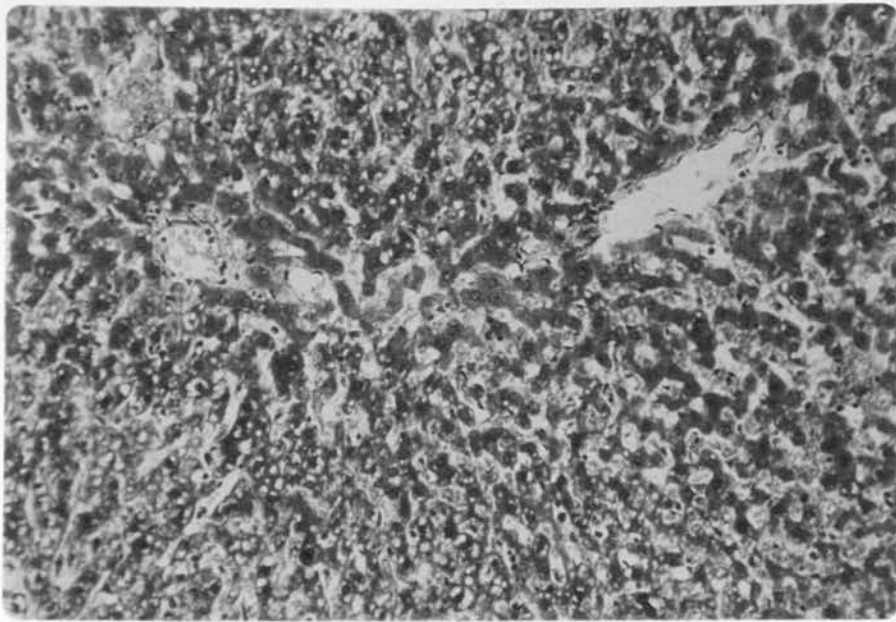


Fig. 32

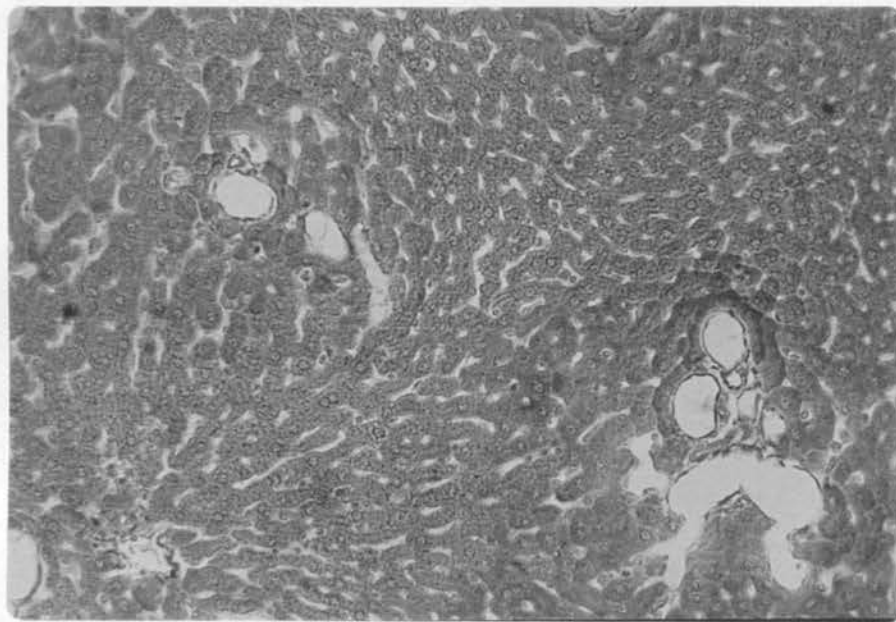


Fig. 33.

Fig 32 and 33 are similarly prepared, Masson stained, paraffin sections of the livers of rats after porto-caval anastomosis. 32 is of a 50 day survivor. The parenchymal cells are smaller than the sham operated but larger than the 21 day survivor. Coarse fat globules were present in centrilobular cells in this individual.

33 is of a 100 day survivor. The cells are larger than the 21 and 50 day survivors, and approximate to the sham-operated animal in fig. 29. In this unselected field the portal radicles are closer than in the normal liver, indicating a reduction in the number of liver cells after porto-caval anastomosis.

(both  $\times 150$ )

|                                   | Normal     | 21 days   | 44 days    | 71 days   | 103 days   |
|-----------------------------------|------------|-----------|------------|-----------|------------|
| Mean Parenchymal Cell Diameter    | 25.2 $\mu$ | 18 $\mu$  | 17.4 $\mu$ | 20 $\mu$  | 24.8 $\mu$ |
| Mean Parenchymal Nuclear Diameter | 13.4 $\mu$ | 9.6 $\mu$ | 10 $\mu$   | 9.6 $\mu$ | 11.6 $\mu$ |

Cell and Nuclear Diameter of Hepatic Parenchymal cells  
in Rats with Porto-caval Anastomosis.

Fig. 34.



fallen weight, would have been considered normal microscopically. There was no fibrosis, no abnormality in fat deposition, no abnormality in histological glycogen content, and normal reticulin network. The parenchymal cells, however, were smaller than average (Figs. 29-33). Whereas in normal livers, a mean parenchymal cell diameter of 25 u was the rule, between 21 days and 50 days average figures of 17.4 u to 20 u were found in a series of animals (Fig. 34). There was no reduction of the number of cells between portal radicles and central veins. After the 50th day the mean cell diameter rose in those animals with increasing body weight, e.g. Rat No. 42 had mean cell diameter of 24.8 u, had survived 103 days, and its body weight had risen 30% from a low of 60% of its pre-op. weight.

#### INFERENCE:

The inference is drawn that following diversion of portal blood past the liver, there is a reduction of liver mass by approximately 60% to a new low mass which is maintained in proportion to the animal's subsequent body weight. The reduction of mass is at first at the expense of cell substance and not of cells per se, i.e. it is an atrophy. Later the cellular dimensions return to normal, but there are less cells i.e. there is aplasia.

#### ABNORMALITIES IN HISTOLOGY OF LIVERS

Three rats had severe histological abnormalities.

Rat 30 Killed at 21 days, this showed histiocytic reaction in the portal radicles. The histiocytes contained haemosiderin in their cytoplasm (positive P.B.R.). No other abnormalities were seen. No abnormal fibrous tissue pattern was seen (Figs. 35-37).

Rat 28 Died at 96 days. There was a diffuse fibrosis of the parenchyma without any sign of regenerative or other restorative changes on histological examination. There was a dense infiltration of small round cells in nearly all the portal radicles (Figs. 38 and 39).

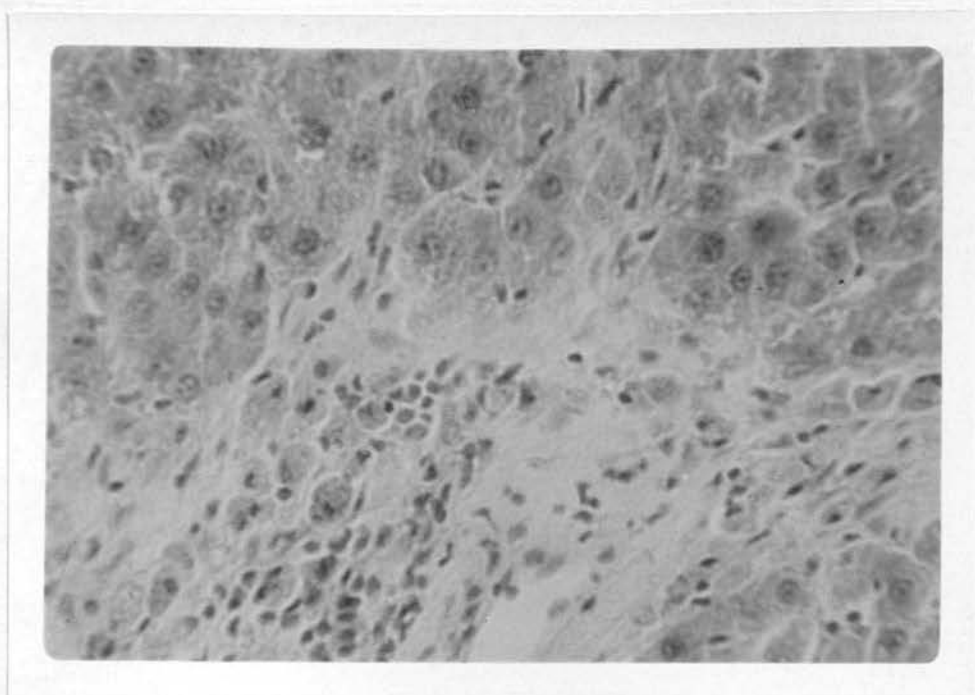


Fig. 35 Histiocytes containing haemosiderin - Prussian blue positive -  
were seen in several animals after operation

(H.E. x 300)

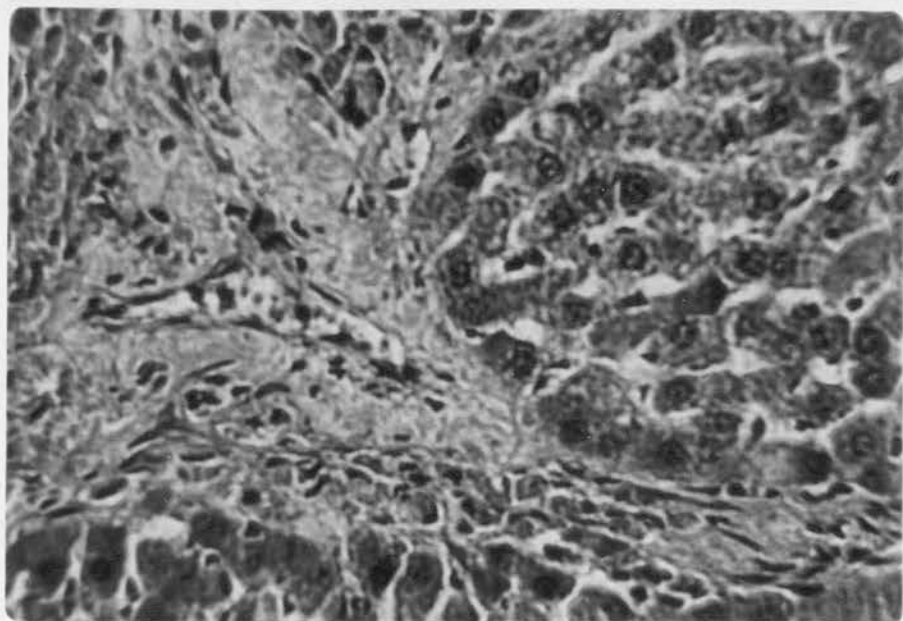


Fig. 36

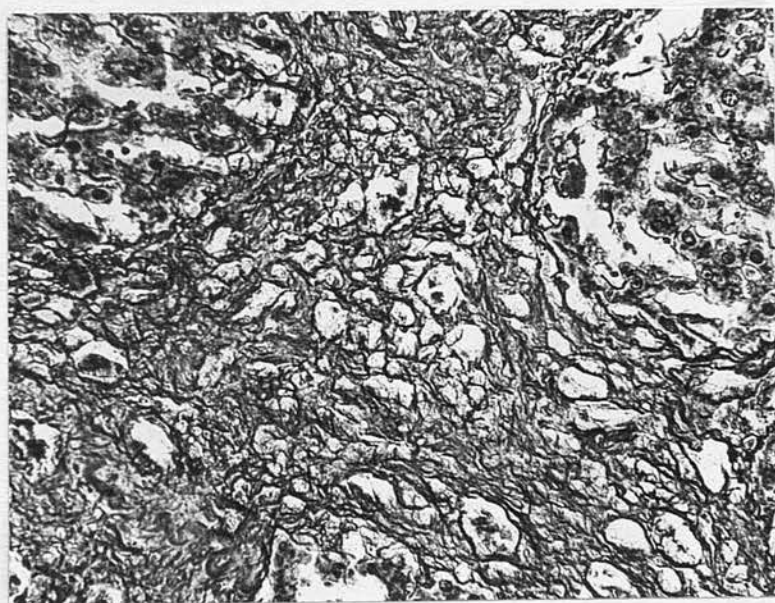


Fig. 37.

Masson and reticulin preparations from livers containing  
haemosiderin-laden phagocytes in the portal radicles.

Fig. 38

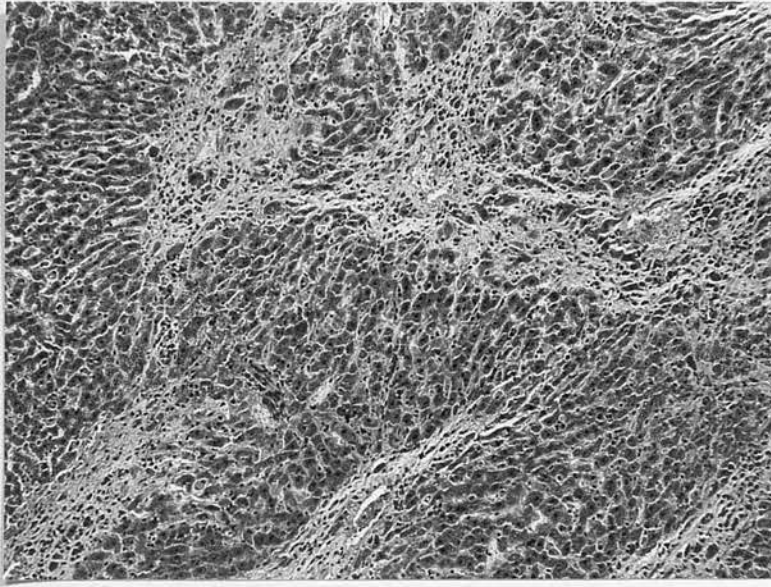
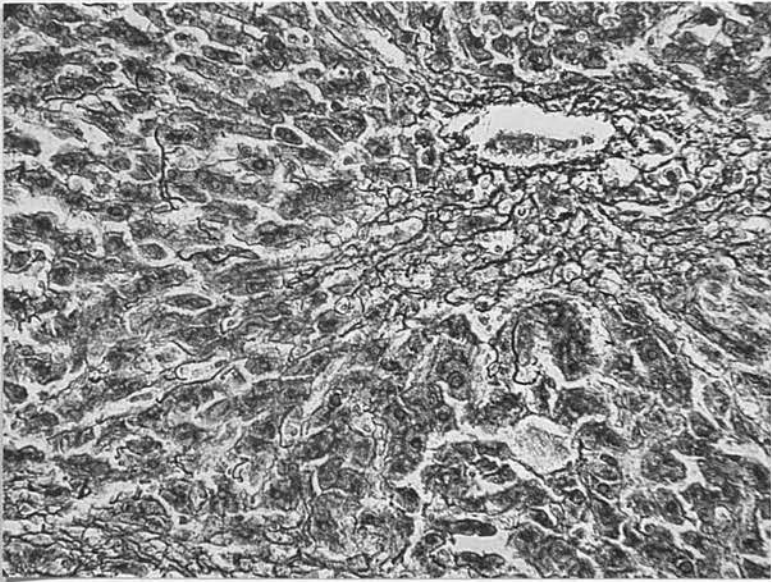


Fig. 39



Masson (above) and reticulin preparations from a rat with portal cirrhosis 60 days after operation.



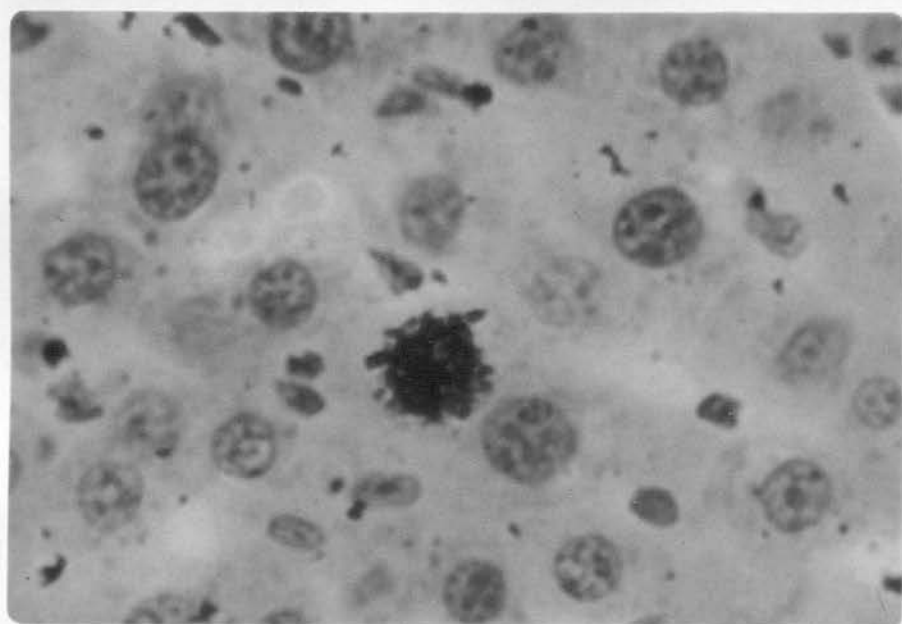
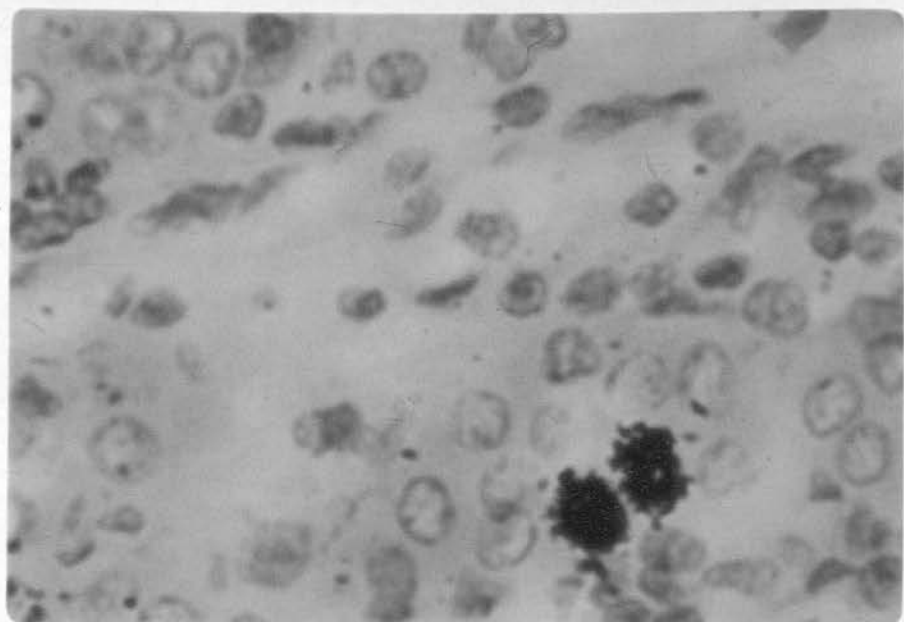


Fig. 40 H+E stained  $^3\text{H}$ -thymidine autoradiographs of liver showing localisation of thymidine in the nuclei. Above, daughter cells presumably formed by division in the 12 hours between giving thymidine and death. Below, a cell preparing for division and/or synthesizing DNA.



Rat 52 Died at 40 days. Broad bands of fibrous tissue are present in one part of the liver. Again no regenerative activity is distinguishable by ordinary microscopy.

#### TRITIATED THYMIDINE STUDY OF LIVERS

Additional information on the behaviour of the hepatic cells is obtained by the use of tritium labelled thymidine.

Thymidine is taken up by cells synthesising DNA, and its incorporation in nuclei is permanent. The tritium acts as a marker of these cells and their daughters after division (Fig.40).

In a normal rat, the rate of turnover of cells seems fairly constant from animal to animal.

There was no difference between the turnover rates for normal and operated animals, as judged from the relative proportions of labelled nuclei.

A large increase was seen in the rat (No. 28) with the diffuse cirrhosis. This feature was displayed in spite of the absence of regenerative features on ordinary microscopy, although mitotic figures in rodent livers are not always easy to ascertain as such.

#### DISCUSSION

Published experimental observations on the changes in normal livers following anastomosis are few (Bellmann, 1926; McMichael, 1938; Whipple, 1943). They are each of a few dogs.

Reliably controlled conditions are at best difficult to achieve in such large animals. Observations on the livers of rats following anastomosis were produced first by Lee & Fisher (1961) and Fisher & Fisher (1962). They showed that there was a severe reduction of hepatic mass, and that no light microscopic changes occurred, save for reduction of the size of parenchymal cells. There were changes at an electron

microscopic level, viz., dilatation of the granular endoplasmic reticulum.

The reduction in size of the cells, with the shrinkage of liver bulk occurs without apparent alteration of the reticulin framework, and it is not associated with cirrhosis. As far as could be ascertained by light microscopy using either P.A.S. or Best's carmine, no detectable deviation from the wide range of normal appearances for glycogen content occurred.

That Whipple and McMichael observed the phenomenon of weight reduction in dogs' livers after anastomosis would tend to indicate that the change may occur in all animals after shunting. There is, unfortunately, no note of the structure of the liver of the animal reported as having a congenital end-to-side porto-caval shunt (Child et al). Sudan stains on frozen sections showed that, although there was a considerable range of the amount of fat in the livers of normal and operated rats, the operated rats had much less fat than normal or sham operated ones. The fat when present in operated rats was in small globules in the periportal cells. It was rare to find fat in centrilobular cells, but it was not uncommon to fail to demonstrate fat at all in some, especially during the second three week period when the body weight was at its lowest. During the first three weeks, large quantities of fat were found, mainly in periportal cells, but with very fine globules occasionally in centrilobular cells.

It was interesting in view of the studies of Dible et al that in spite of the severe anaemia which occurred in some, terminally, these anaemic rats showed no increase in fat in the liver.

The probable explanation is that there was depletion of total body fat and that little was available for deposition in the liver cells in stress. This was the experience of Dible et al who found that emaciated or starved rats failed to show fat globules in the livers in response to carbon tetrachloride.

The weight loss of these livers was not considered to be due to depletion of stainable fat, because of the similar range of amount of fat in control animals. It could be, of course, that naked fat had been depleted too and was not available for phanerosis. The search for kidney changes is reported later.

Why should reduction occur at all? As mentioned above, all the substances normally handled by the liver should reach it in the arterial perfusion unless they are extracted by other chemically competent tissues, e.g., possibly the kidneys or brain. If such an adaptation occurred perhaps a hypertrophic or hyperplastic response might be found in some other tissues. Also, if adaptation of this sort occurred, it could underly the phases of body weight loss, stability and the recovery which could indicate mastery of the situation.

Control of hepatic blood flow has been studied in many animals (Deysach in Child 1954) and in amphibians and mammals it seems a general rule that oxygen is carried to the liver in the relative proportions 3 : 5 by arterial and portal venous lines. There is evidence that there is active neural participation in the control of flow and stimulation of splanchnic nerves alters the pattern of flow through the liver as a whole, increasing central flow and rate of flow, diminishing peripheral flow and elevating portal venous pressure (Daniel & Pritchard). There is also active control of the gut arterioles and hepatic artery in exercise and after meals. The

The whole portal system is delicately balanced and controlled. After ligation of the portal vein in dogs the hepatic arterial flow has been shown to increase (Child). If such an increase occurred in rodents then the loss of liver mass would be less than 60% if oxygen availability alone were the factor.

No histological changes are previously reported in the kidneys in this situation, although such conditions as the hepatorenal syndrome occur, and a variety of amino-aciduria's are associated with liver disease, most notably with Kinnear Wilson's disease. The search for kidney changes is reported later.

Cerebral changes are discussed later.

PONTO-BASAL GANGLIA NEUROPHALOSIS

A phenomenon of stupor or coma was observed as a terminal event in 5 of these animals.

This state was observed first in a rat thought to be dead. On opening the cage, however, it stood up slowly and staggered round the cage, stood unsteadily for a few moments and then fell down again. It continued to breathe slowly, and responded to touching by head and weak limb movements only for 12 hours, before becoming comatose and dying 16 hours after the abnormality was first noticed.

Four other rats **NERVOUS SYSTEM** in similar conditions, with the duration of                      between six and thirty-six hours.

There was no tremor. All had bled into the gastro-intestinal tracts from gastric erosions, and altered blood was present in their small intestines. There were no anoxic histological features in the brains of these animals.



### PORTO-SYSTEMIC ENCEPHALOPATHY

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Four other rats have been found in similar conditions, with the duration of the episode being between six and thirty-six hours. There was no tremor. All had bled into the gastro-intestinal tracts from gastric erosions, and altered blood was present in their small intestines. There were no anoxic histological features in the brains of these animals.

The following features were systematically evaluated in all brains, test and control:-

#### Morphological features of Astrocytes - Number

Size

Shape

Staining characteristics

No. of astrocyte fibres

Presence or absence of  
nest bodies

or positive P.A.S. intranuclear inclusions.

## CEREBRUM

### Introduction and Methods

The brain of a rat grows rapidly in the first 10 post natal days, rising from 0.2 to 1.0 G. From 10 to 80 days a less rapid increase occurs in a linear fashion to an adult weight of 1.8 - 1.9 G. Thereafter brain weight increases only slightly - 400 mg. in 150 days (Donaldson, H.M., Craigie, Sugita-in Craigie). Cortical thickness does not increase appreciably after 80 days.

The smallest rat included in the series was approximately 80 days old, and all others were over 120 days.

It has not been possible to find any reference to the fine histological changes which may occur in the ageing rodent's brain. The greatest age achieved in the whole series was 380 days, and the distribution of brains by age is shown below. The mean age at death was 185 days. Control, normal animals of comparable ages were studied to properly evaluate any changes which might result from advancing days.

With these considerations in mind the histological findings in the series were plotted against age of animal (Figs. 76 & 77) and against duration of survival of operation, either sham, or proper.

The following features were systematically evaluated in all brains, test and control:-

Morphological features of Astrocytes - Number

Size

Shape

Staining characteristics

No. of astrocyte fibres

Presence or absence of  
Best Carmine

or positive P.A.S. intranuclear inclusions.

Oligodendrocytes - number  
 Microglia - presence or absence  
 Neurones - Number  
                   Chromatolysis  
                   Opalski cells  
 Myeline - Myelopathy  
                   Tract degeneration

#### Microcavitation.

The following three principal conclusions emerged:

1. The only changes which did occur, were changes in size, shape and staining properties of astrocytes.
2. No Best Carmine or P.A.S. positive intranuclear inclusions were seen.
3. There was no evident increase of astrocytic fibres.

None of the other features listed were found to be abnormal, and abnormal features did not occur.

Attempts to quantitate the cell types were abandoned because of the absence of any difference in simple counts of relative cell proportions between normal and long-surviving test rats.

#### ASTROCYTIC NUCLEAR MORPHOLOGICAL CHANGES

The changes which were observed were increase of size of some of the astrocytic nuclei, increasing irregularity of their outline and pale staining with ordinary stains. The earliest or slightest change seen was the change of some nuclei from circular to oval or reniform outline. Some such cells can be found in normal brains, but the ease with which they could be found determined whether the sections were graded 0 or  $\frac{1}{4}$ . With obvious indentation of the nucleus, even although the size was not obviously increased, pallor of staining was observed.

In normal rats there is difficulty in distinguishing

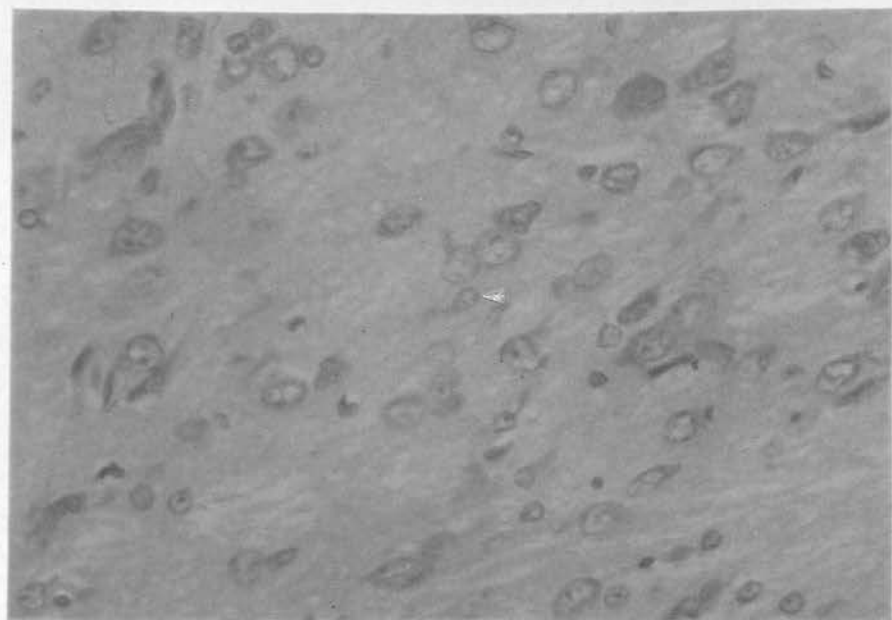


Fig. 41 Sham-operated rat - H+E section of nuclei pontis.  
 The oligo-astrocytic nuclei have round, relatively uniform nuclei.  
 (x 750)

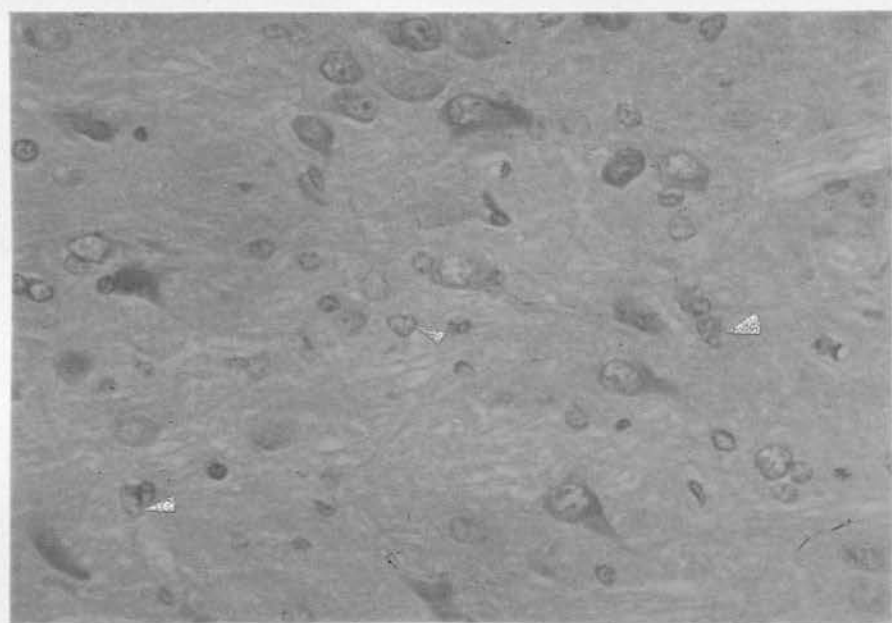


Fig. 42 63 days after ponto-caudal anastomosis - H+E section of  
 nuclei pontis. Several oligo-astrocytic nuclei appear larger than  
 average, have irregular outlines, and stain less than those in fig. 41.  
 (x 750)

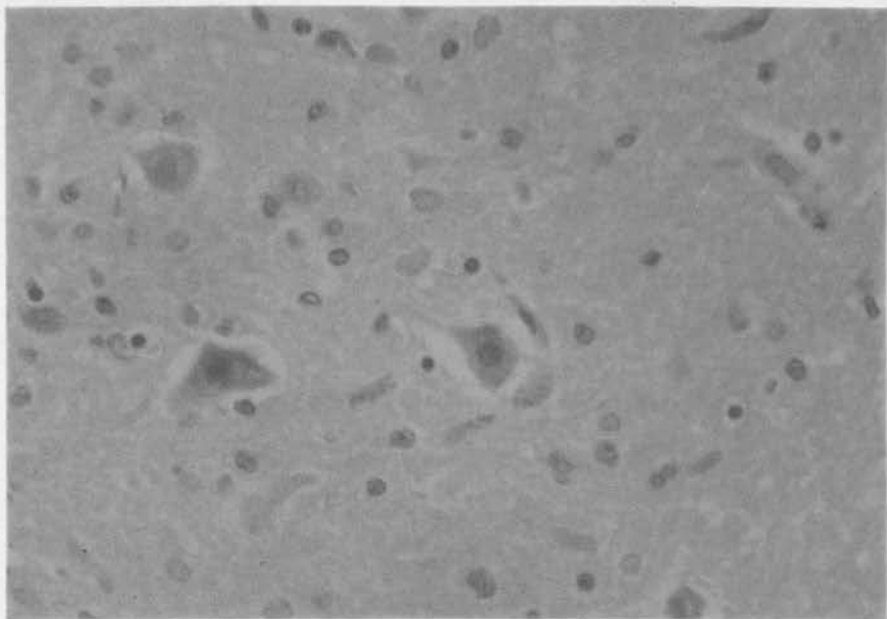


Fig. 43 56 days after porto-caval anastomosis - H+E section of dentate nucleus. Several of the oligo-astrocytic nuclei appear larger than average, have pale nuclear staining, and have folded or indented nuclear membranes.

(X 750)

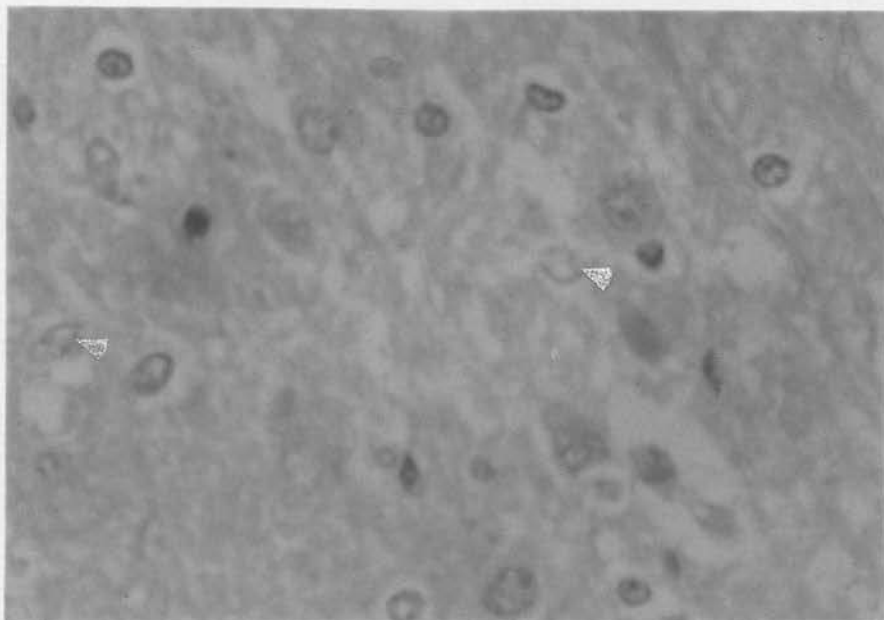


Fig. 44 62 days after porto-caval anastomosis, H+E section of dentate nucleus. Several of the oligo-astrocytic nuclei have the characteristics of Alzheimer's type II cells.

(X 1200)



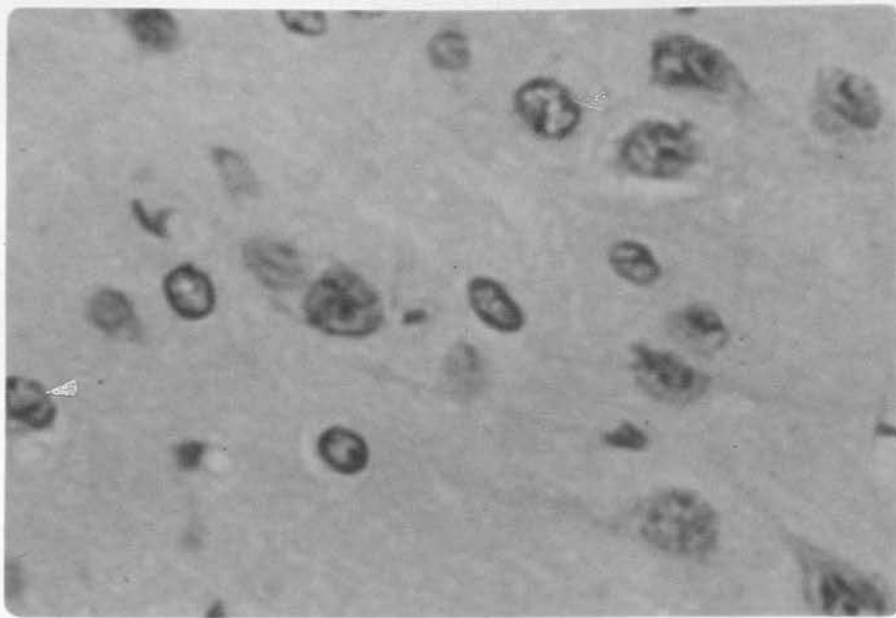


Fig. 45 67 days after porto-caval anastomosis — H+E section of dentate nucleus. Two irregularly-shaped glial nuclei.

(x 1500)

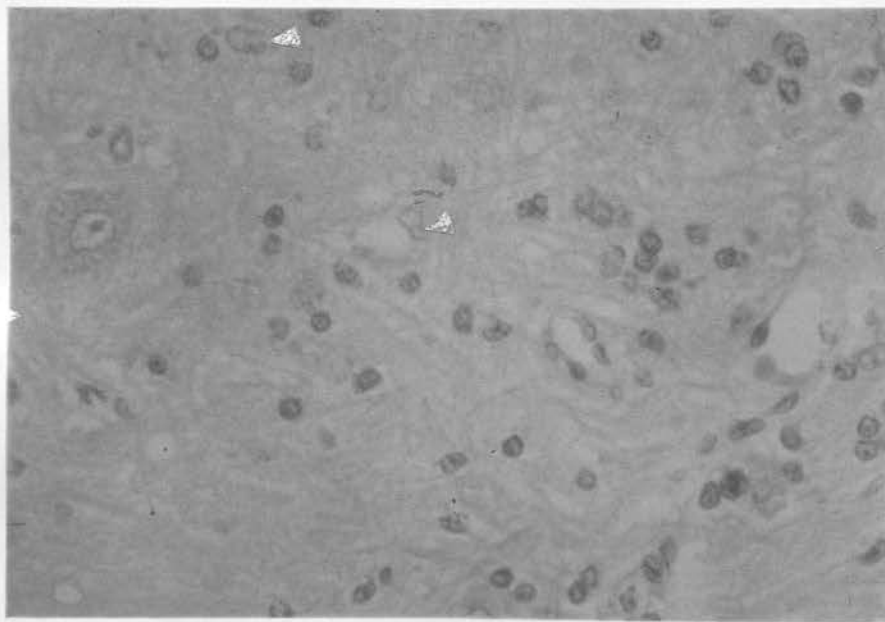


Fig. 46 103 days after porto-caval anastomosis — H+E section of dentate nucleus. Several oligo-astrocytic nuclei, among many normal-looking, are enlarged, pale staining, and have folded membranes. They have the characteristics of Alzheimer's type II astrocytes.

(x 750)

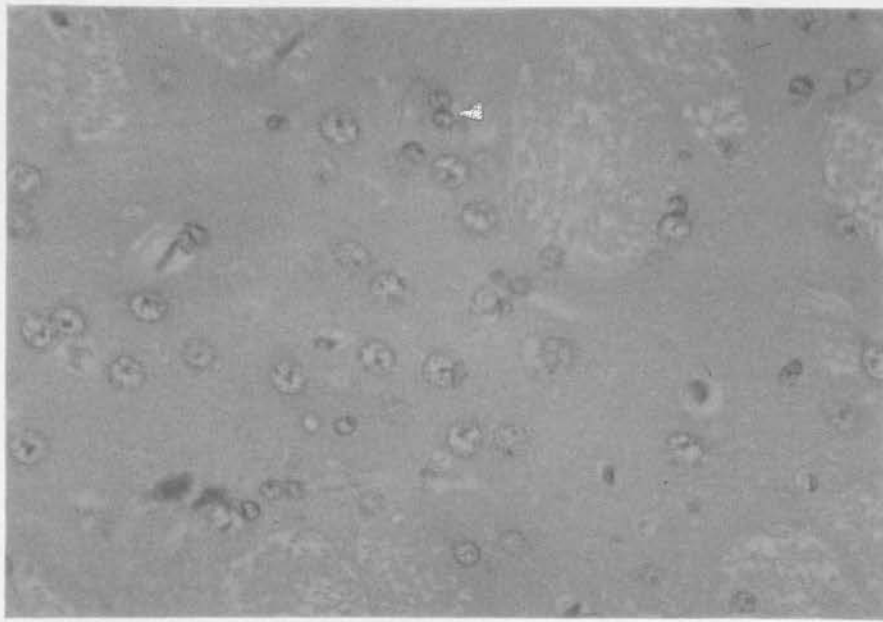


Fig. 47 Sham-operated rat, 80 days after operation. H+E section of thalamus. The oligo-astrocytic nuclei are of regular, round size and shape.

(X 750)

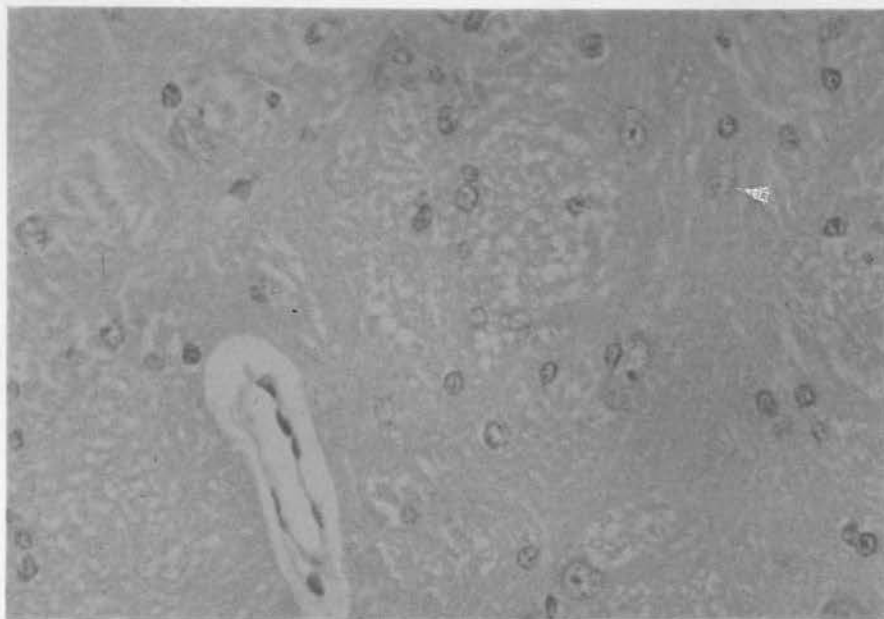


Fig. 48 87 days after porto-caval anastomosis - H+E section of thalamus. Several oligo-astrocytic nuclei are irregularly shaped and pale.

(X 750)

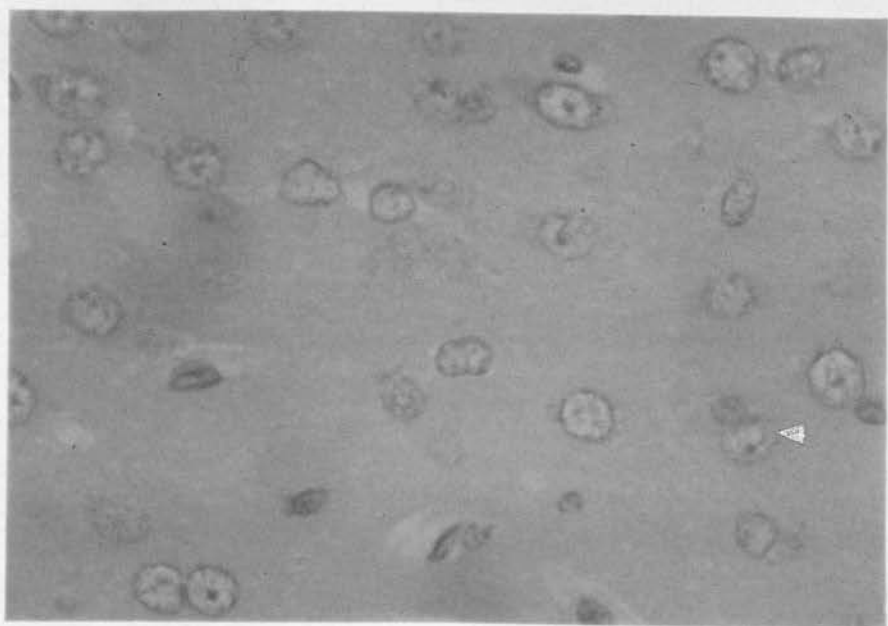


Fig. 49 96 days after porto-caval anastomosis. - H+E section of thalamus, with irregularly shaped oligo-astrocytic nuclei.  
(x 1200)

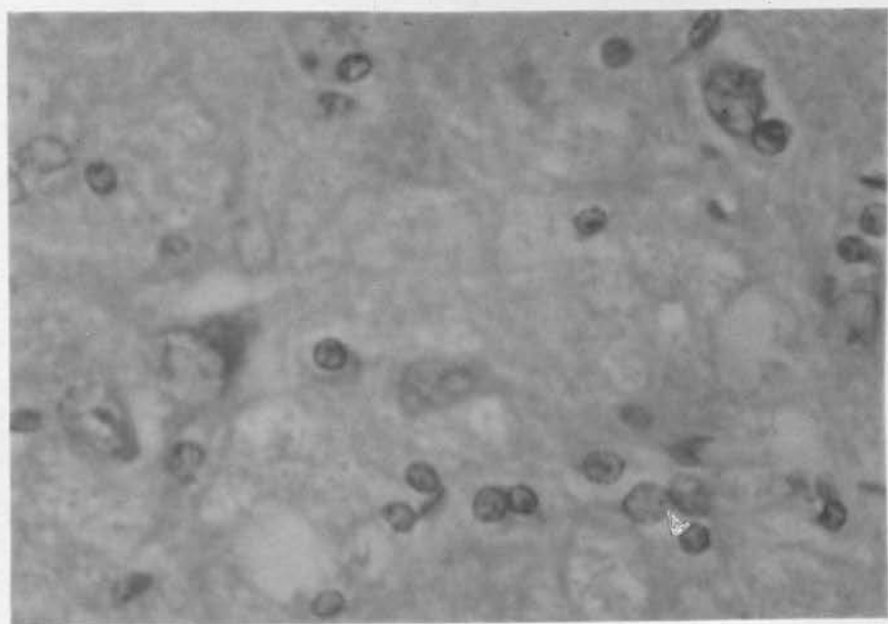


Fig. 50 225 days after porto-caval anastomosis - H+E section of thalamus, with irregularly shaped oligo-astrocytic nuclei.  
(x 1200).

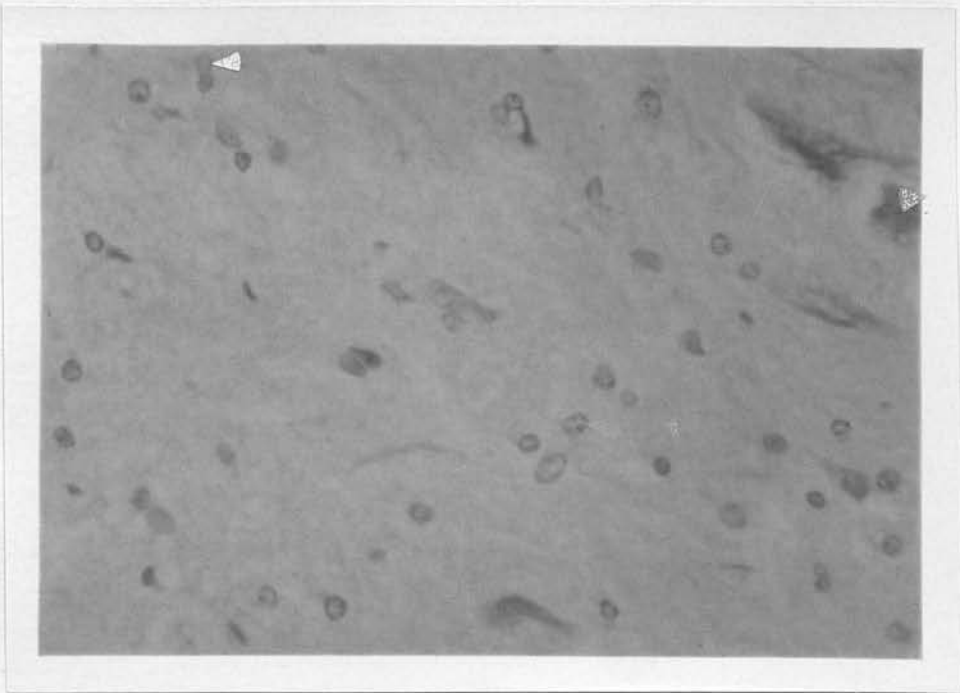


Fig. 51 103 days after ponto-caudal anastomosis - H+E section of 6th nerve nucleus. Irregularly-shaped oligo-astrocytic nuclei.

(x750)

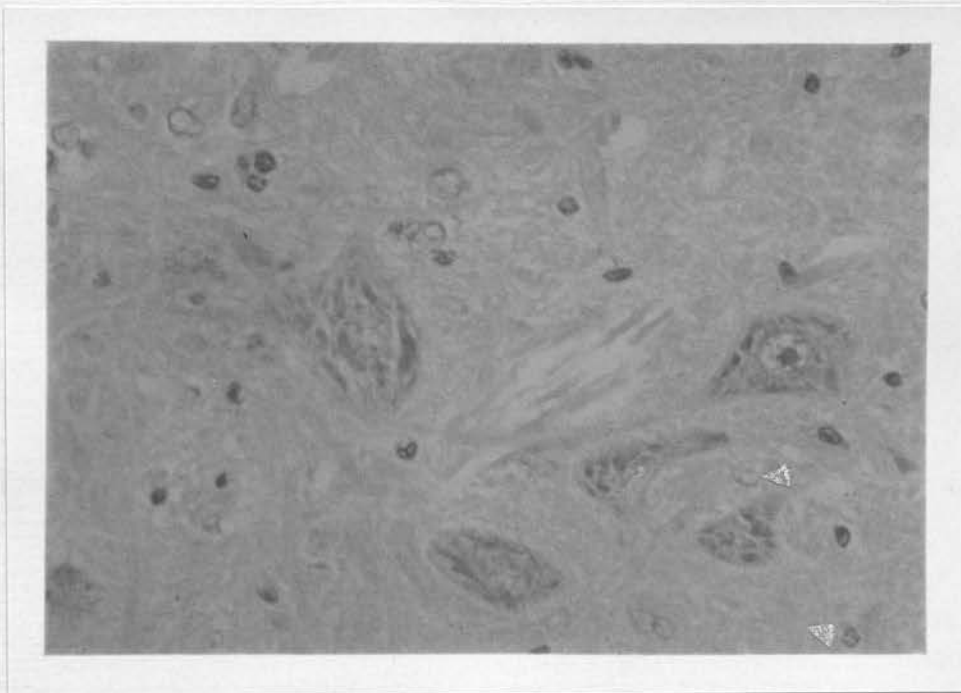


Fig. 52 225 days after ponto-caudal anastomosis - H+E section of anterior horn of spinal cord. Irregularly shaped oligo-astrocytic nuclei.

(x750)



oligodendrocytic and astrocytic nuclei. The problem has caused the glial nuclei to be labelled oligo-astrocytic if the features for rigid classification as one or the other are absent. The oligodendrocytic nucleus is round, and stains darkly, having dispersed D.N.A. It has no nucleolus, like with the astrocytic nucleus, which is slightly larger, and has more dispersed D.N.A. which is often clumped at the nuclear membrane (Smart & Leblond).

The reniform nuclei were labelled as astrocytic because they were slightly larger looking than the oligo-astrocytic or oligodendrocytic, and had the astrocytic dispersal of D.N.A.

Another difficulty which arose was of distinguishing the nuclei of neurones from astrocytes, especially where the neuronal nuclei were small and the cytoplasm scanty or difficult to stain. If a nucleolus was present the nucleus was considered neural.

When the astrocytic nuclear outlines were frequently found to be irregular and pallor of staining was a prominent feature of many, the sections were placed in a category labelled ( $\frac{++}{+}$ ) (Figs. 41, 43, 44 & 45). The fully developed Alzheimer type 11. nucleus was present in those classed as ( $\frac{+++}{+}$ ) (Figs. 42, 46 - 52).

Four grades were chosen (0,  $\frac{+}{+}$ ,  $\frac{++}{+}$ ,  $\frac{+++}{+}$ ). All sections were examined blind on two separate occasions. The abnormal features were sought in cerebral cortex, central cerebral nuclear mass (basal ganglia), brain stem, dentate nuclei, cerebellar cortex and spinal cord.

Abnormalities beyond range  $\frac{+}{+}$  were never seen in cerebral cortex or junction of cortex and white matter.

The most severe changes were in the astrocytes of the dentate nuclei, basal ganglia and brain stem nuclei, in descending order of severity. Alzheimer 11 cells might only be found in the dentate nucleus in one case, dentate and basal ganglia in one more severely affected, and in all three



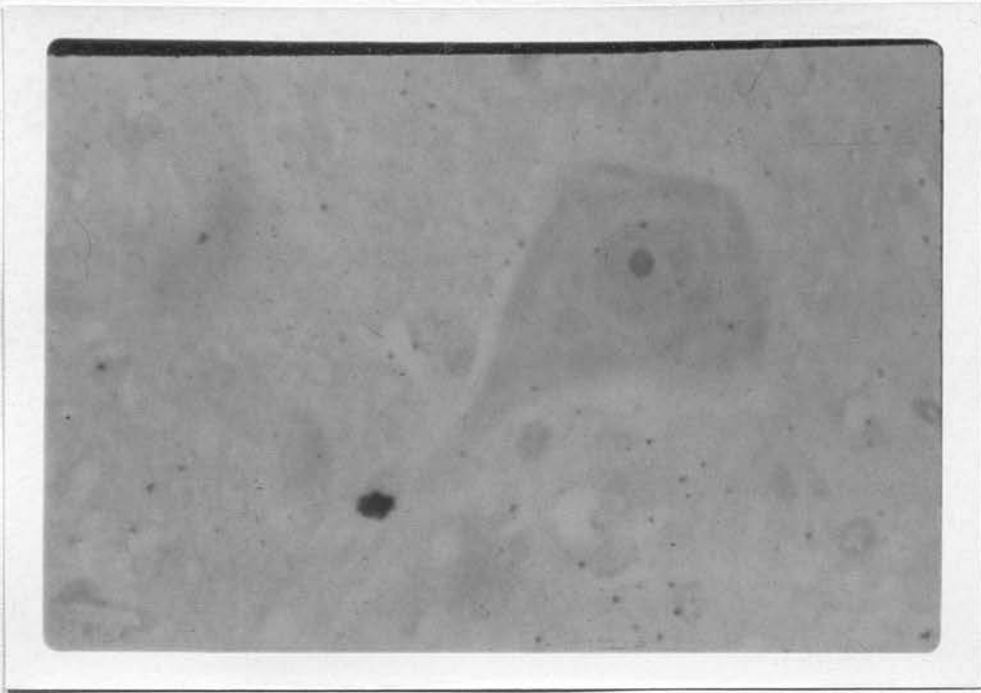


Fig. 53 Anterior horn of spinal cord, 100 days after porto-caval anastomosis. Dense labelling with  $^3\text{H}$ -thymidine has occurred in an unidentifiable nucleus.

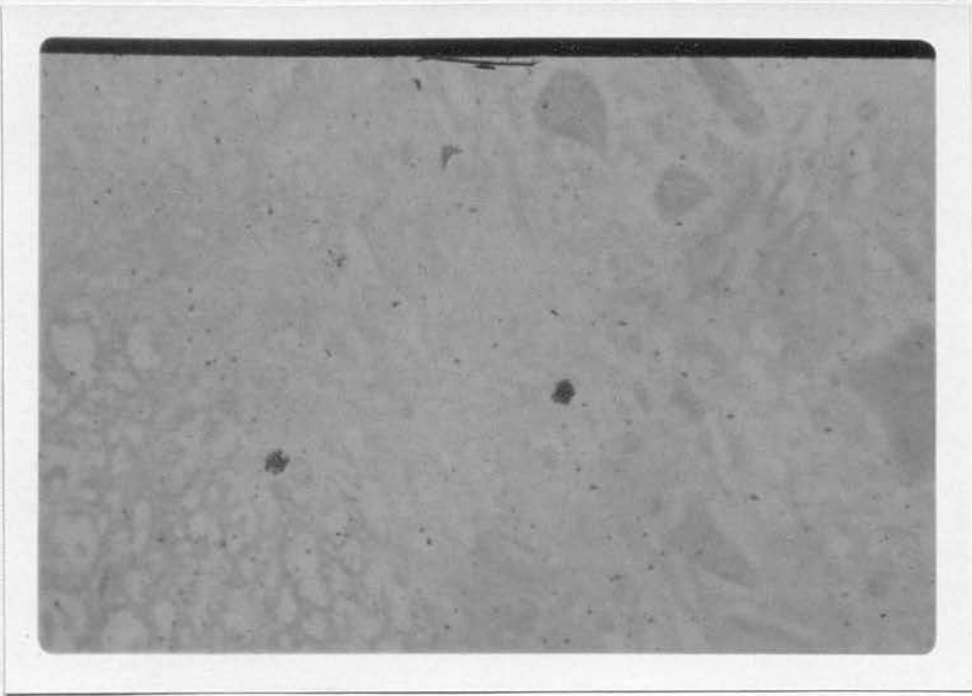


Fig. 54 Spinal cord - anterior horn and antero-lateral white matter. 100 days after porto-caval anastomosis. Two unidentifiable nuclei heavily labelled with tritiated thymidine.

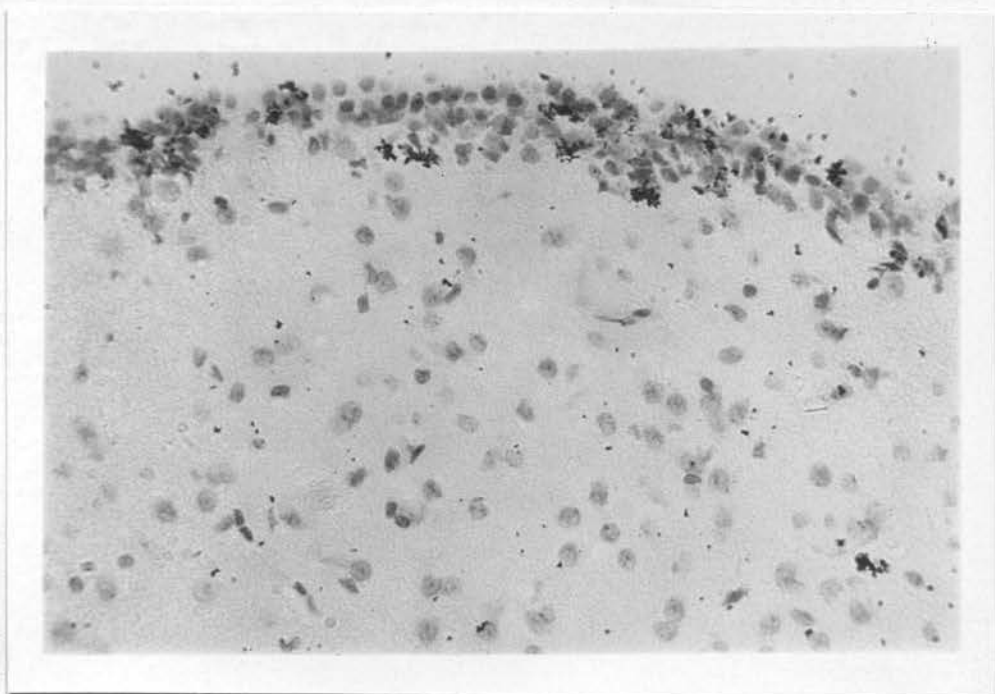


Fig. 55 DNA synthesis shown by  $^3\text{H}$ -thymidine labelling of nuclei of the subpial glia of the lateral ventricle in a normal rat of 350 days.

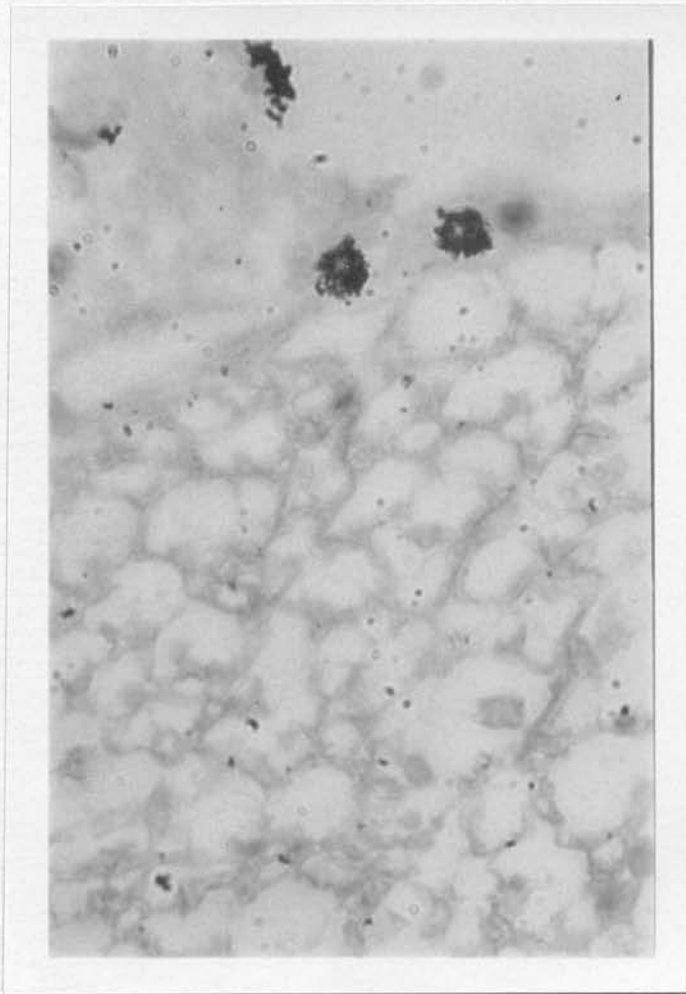


Fig. 56 Two  $^3\text{H}$ -thymidine labelled nuclei in the spinal cord of a rat of 350 days. These nuclei are probably the results of division during the interval between giving thymidine and death, a period of 16 hours in this case.



regions in a severely abnormal one.

All rats scoring  $\frac{++}{+}$  or  $\frac{+++}{+}$  were re-examined blindly for a third time and regraded if necessary although it was only found necessary to change a grade on two occasions.

The results of these observations are presented in Figs. 76-77 where 0 and  $\frac{+}{+}$  include the normal range of appearances and  $\frac{++}{+}$  and  $\frac{+++}{+}$  are grades of abnormality. They demonstrate that significant abnormalities in the morphology of astrocytes only occurs after 55 days of porto-systemic shunting. The changes are found only in animals surviving the proper operation for this time, and are not seen in sham-operated rats of similar survival, or in normal animals of the same or greater age.

#### AUTORADIOGRAPHIC EXAMINATION OF SECTIONS OF NEURAL TISSUE

The systemic application of 0.5 m.c. tritiated thymidine 12 - 24 hours before killing the test animals was intended to challenge the hypothesis that the reacting astrocytes (grade  $\frac{++}{+}$ ) and Alzheimerforms (grade  $\frac{+++}{+}$ ) are products of, or are preparing for, division (Lapham, 1961).

No definite labelling of astrocytic nuclei in the substance of the cerebral hemispheres, cerebellum or brain stem was seen after 44 days. Labelling was seen in a few nuclei in the cords of animals which had survived the operation up to 220 days, but the nature of the nuclei could not be ascertained (Figs. 53 & 54). Labelling was also seen in the subependymal glia of the walls of the lateral ventricles (Fig. 55) in all but one of the rats examined; test animals of over 300 days and control animals of up to 200 days. Labelled cells were occasionally seen in pia mater (Fig. 56).

#### DISCUSSION OF CEREBRAL CHANGES

The finding of abnormal astrocytes bearing the characteristics of reactive forms and Alzheimer's type 2 cells in rats surviving

porto-caval anastomosis by 55 days or more is an advance in the study of these cells. They have only previously been described in rodents by Lapham who found them as early as three weeks after the commencement of a programme of carbon tetrachloride injections to produce cirrhosis in rats. In this study they occur in the presence of a small, histologically normal liver, and their appearance seems to be related to the porto-systemic shunting of blood, associated with plasma ammonium elevation, and not necessarily to functional incompetence of the liver.

The absence of Alzheimer's type 1 nuclei is in accord with one's experience of human liver disease cases where the cell has never been seen, and with the extreme difficulty with which such cells can be distinguished even in older brains from hepatolenticular degeneration. Alzheimer's type 1. illustration bears some resemblance to a degenerate neurone, and it may turn out not to be an astrocyte at all.

No evidence for preparation for division of astrocytes in the substance of neural tissues was gained from autoradiographic studies. No labelled cells were seen in the situations where reactive glial changes were most prominent in brains of rats up to 200 days after operation. This evidence, and the absence of mirror-image and double forms is at variance with the results of Lapham. He based his studies on morphology as has been done here, and surveyed his type 1, type 2. and daughter double forms by Fuelgen microspectro-photometry. He found that type 1. cells were polyploid and the others diploid. From the relative numbers of each type of cell in relation to time in the course of his study he deduced evidence for synthesis of D.N.A. by astrocytic nuclei in the parenchyma of the nervous system and their subsequent division.

In the rats with porto-caval anastomosis no type 1 or daughter

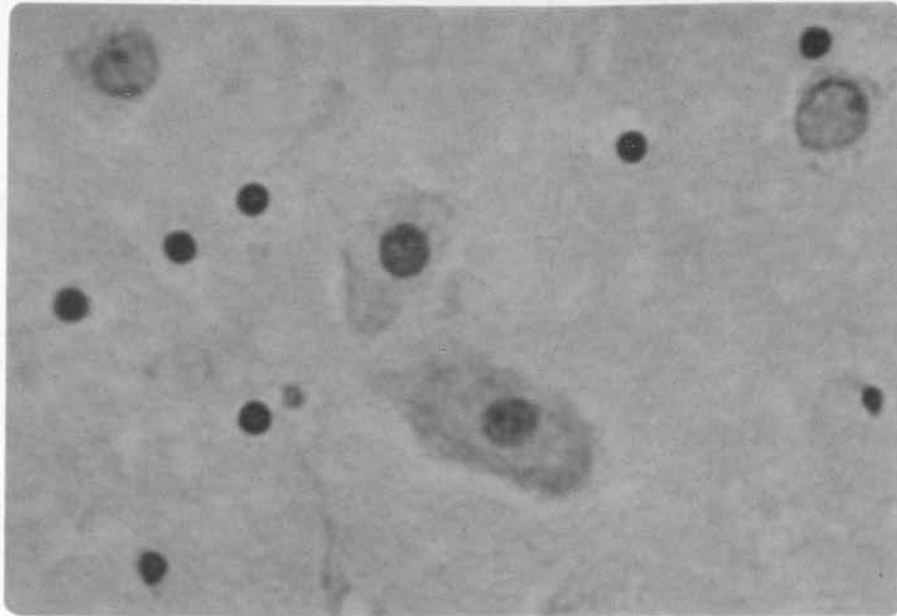


Fig 57  
(H.E. x 1200)

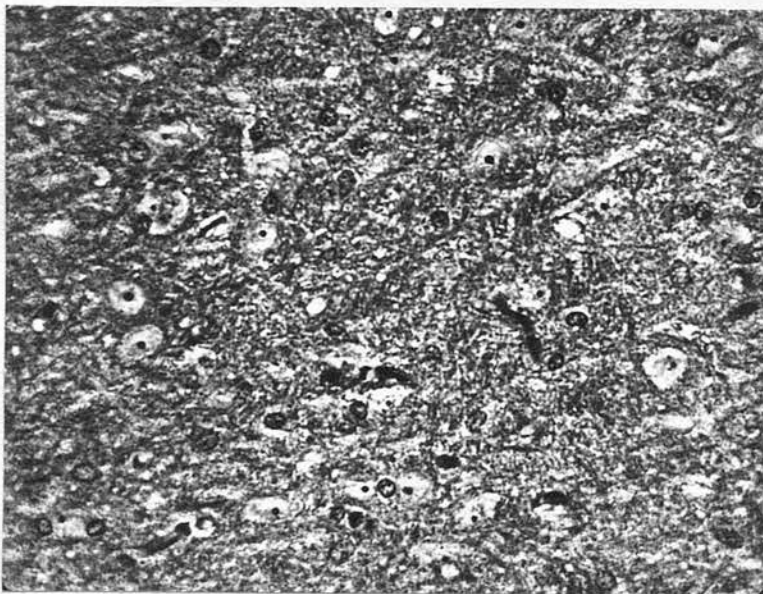


Fig. 58  
(H.E. x 250)

Sections of dentate nuclei from normal rats showing the autolytic changes which result from a period of 24 hours at room temperature after death.

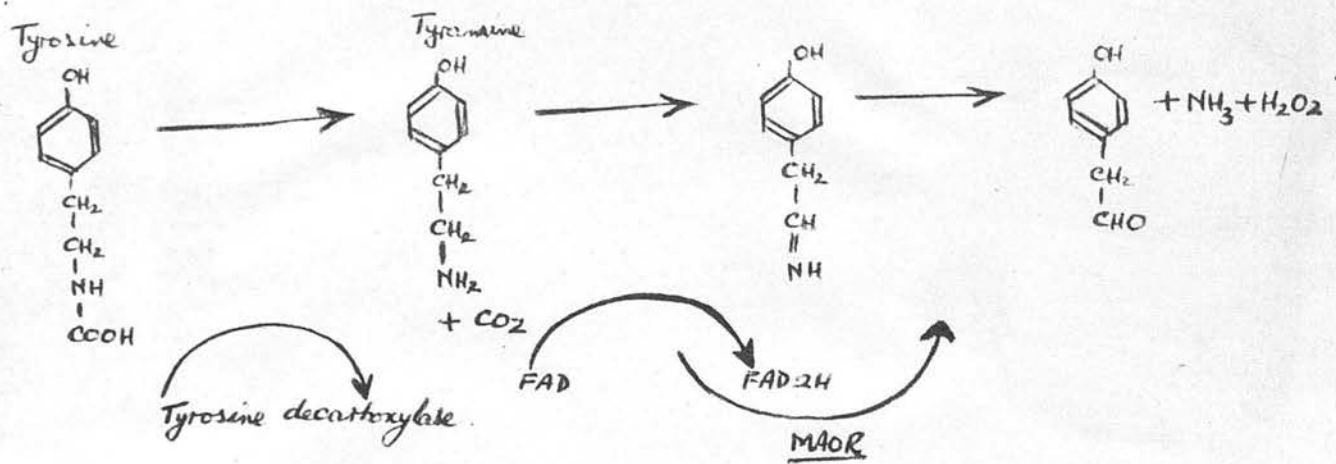
nuclei are found. The failure to demonstrate thymidine uptake by the single dose technique after 200 days could be accountable if the rate of uptake into astrocytes were very slow - much slower than any other cell group so far studied - if it were prevented by the capillary-astrocyte barrier from entering the cell, or if no D.N.A. synthesis were proceeding. The evidence against the first two probable interfering factors is contained in the numerous studies of autoradiography of developing or injured rodent brains (Smart and Leblond), and by the fact that labelling of the subependymal glia of the walls of the third ventricle has continued to 220 days.

Subependymal glial labelling is said by Altmann to occur only in young rodents - up to the 20th week of life. In sham-operated rats it was seen as far as it was followed - to 200 days. In test rats, it was seen at 320 days. Both ages are very much older than would be expected from Altmann. The deductions are that, in the rat, division at this site proceeds well into adult life and there is no apparent difference in the rate of division between test and control animals. Smart and Leblond (1961) and Altmann (1964) hold that there is a constant turnover of oligo-astrocytic cells both from division in situ of cells in the depths of the brain and from migration of cells formed by division of the subependymal glia. (To illustrate that no confusion with autolytic changes occurred, Figs. 57, 58, have been included).

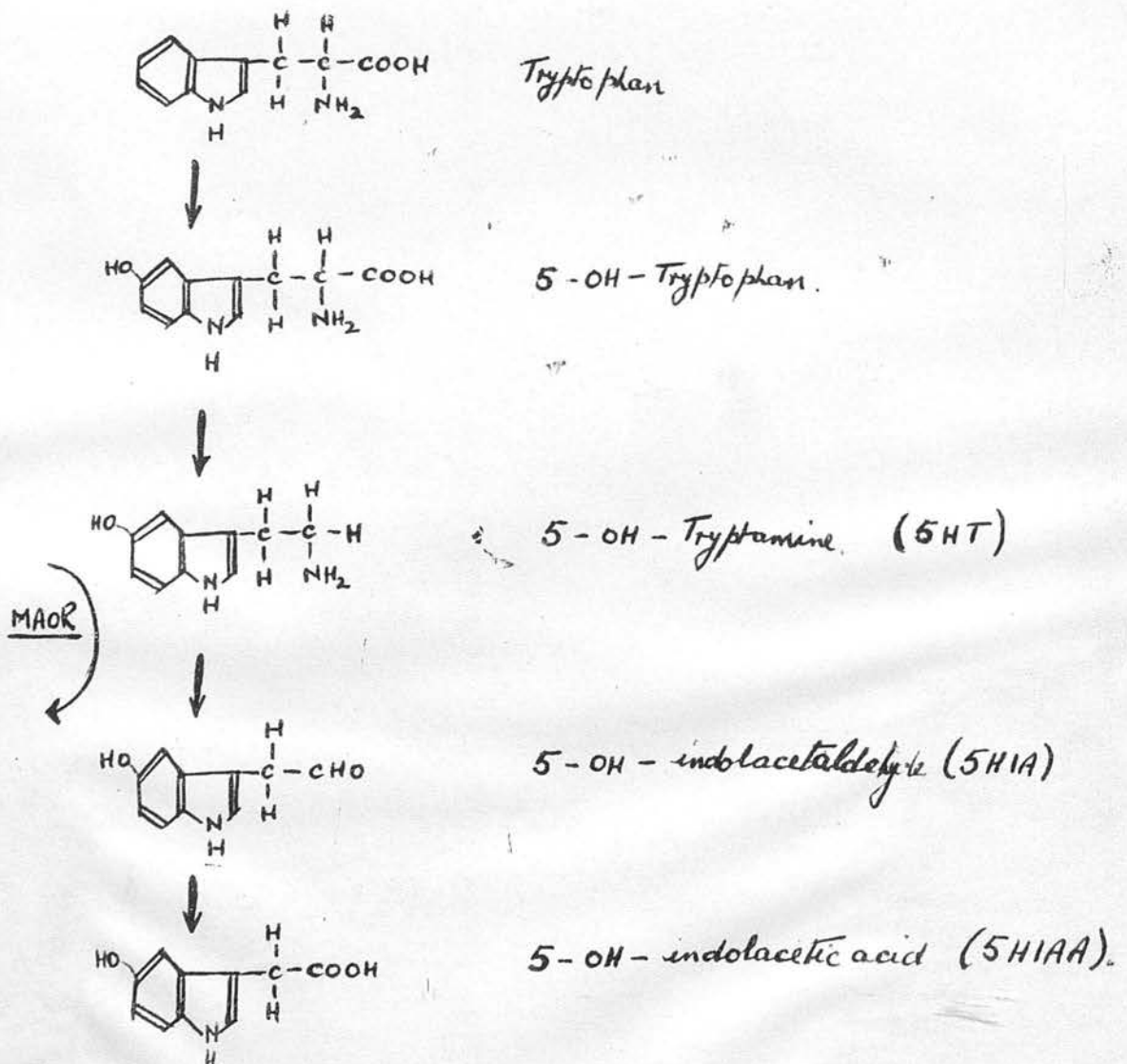
CEREBRAL MONOAMINE OXIDASE ACTIVITY

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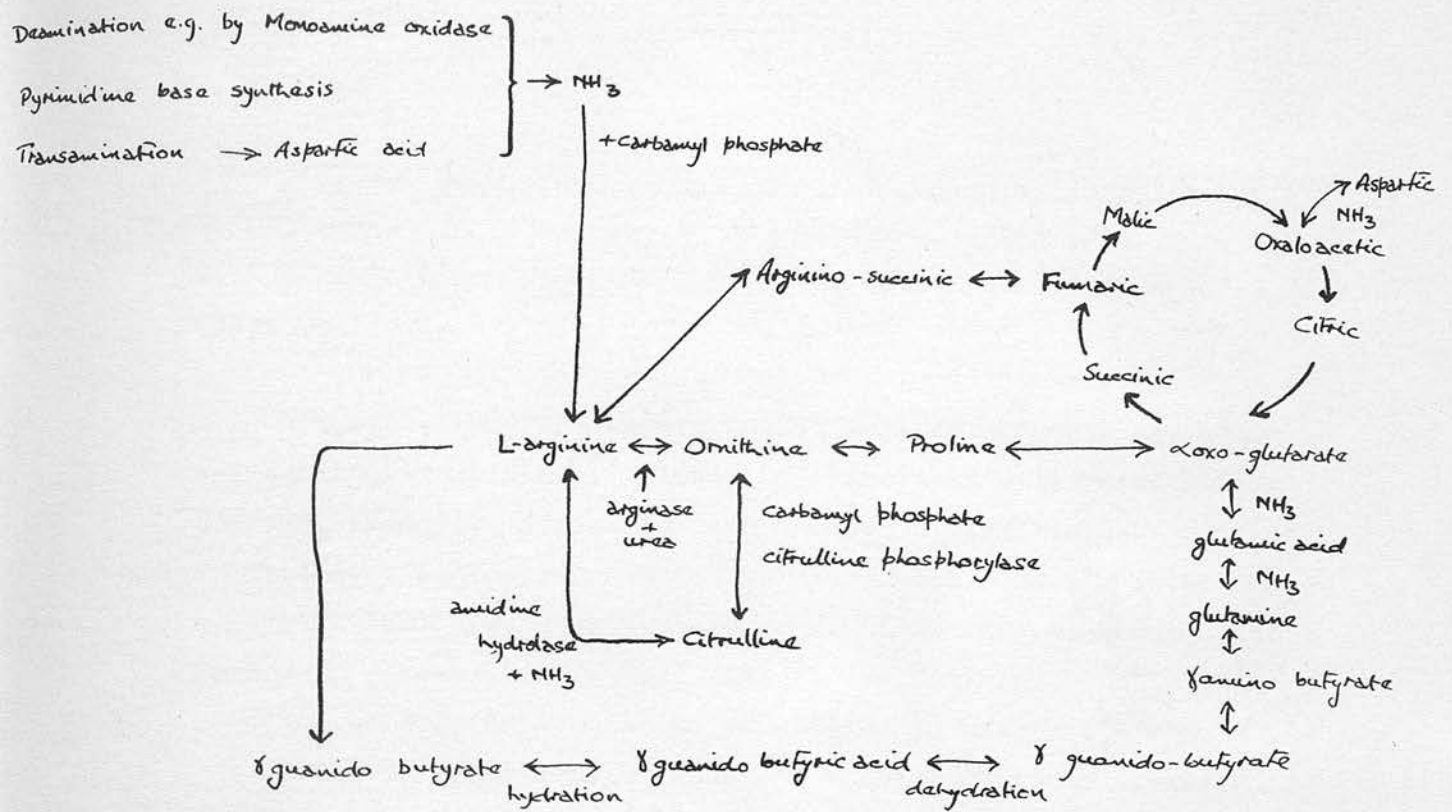




1. Role of Monoamine oxidase in Degradation of Tyrosine.



2. Role of Monoamine oxidase in Metabolism of 5 hydroxy tryptamine.



LL<sub>3</sub> SITES OF APPEARANCE IN INTERMEDIARY METABOLISM, IN RELATION TO THE ORNITHINE AND CITRIC ACID CYCLES.

Fig. 60.

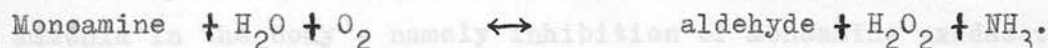
### CEREBRAL MONO-AMINE OXIDASE ACTIVITY AFTER PORTO-CAVAL ANASTOMOSIS

1. Diagram for liberation of  $\text{NH}_3$  by MAO activity (Fig.59).
2. Diagram of important sites of  $\text{NH}_3$  action on intermed. metabolism (Fig. 60).

Monoamine oxido-reductase (MAOR) acts on primary, secondary and tertiary monoamines. Among its important substrates in the body are tyramine, adrenaline and 5 - hydroxytryptamine. It is inhibited by monoamines similar to these, but commonly with  $\alpha$ -methylation (Ergebnisse). It is found in largest quantities in liver, kidney (but not rat kidney) and brain.

In brain it is in inverse proportion to cholinesterase.

Among the functions of MAOR are - Destruction of Adrenaline,  
Deamination of 5-HT,  
Detoxication of amines from  
intestines.



This represents a simplification of the general action of monoamine oxidase. Two illustrations of its position on metabolic pathways are shown in Fig. 59. It is possible that copper acts as a co-factor for monoamine oxidase (Walshe J.M. - personal communication 1963)

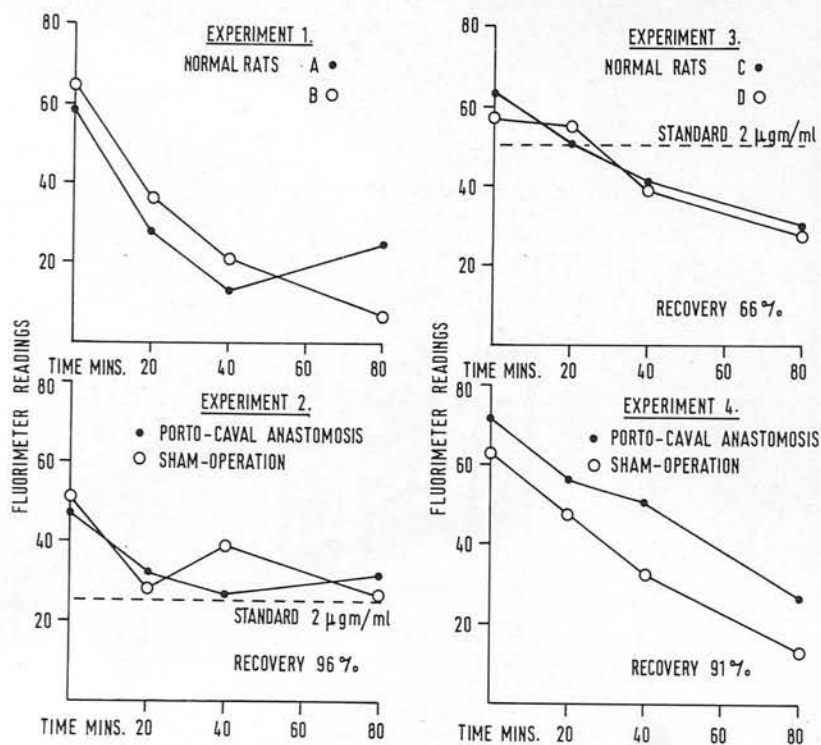
It has long been appreciated that ammonia, or ammonium ions may inhibit certain reactions which normally release ammonia. These reactions may be steps on important pathways of metabolism and their interruption, or retardation may have serious consequences for the body, the tissues or cells most involved. For example (Fig. 60) ammonium ions could interfere at three separate points with compounds directly involved with the citric acid cycle, and it could interfere with the operation of the Ornithine-urea cycle (White et al 1955, von Thoai 1965).

Ammonium ions have been considered to have importance in the pathogenesis of hepatic encephalopathy, although the relationship between the concentration of circulating ammonia nitrogen and encephalopathy is not simple. Some people in deep coma with all other signs pointing to liver coma, do not have elevated circulating levels of ammonia.

Reduction of ammonia level is however an objective in the standard management of patients in coma. The ions are reduced by preventing formation in the bowel by bacterial action or possibly diminishing absorption from the bowel (Ashcroft - personal communication, 1965) by the use of an oral antibiotic neomycin. Reduction of the protein precursors of ammonia in the diet or stopping of bleeding into the gut by surgical intervention, Sengstaken tube pressure, or by pitocin are also used. Ion exchange resins, binding kations have also been used.

In 1957 Sherlock and Dawson published a short communication on a study of another method of reducing the manufacture of ammonia in the body - namely inhibition of monoamine oxidase. Iproniazid (isopropyl isonicotinic acid hydrazide) was given intravenously in a dose of 5 - 10 mg. per kg. body weight, to 17 patients with liver disease. They found that in those eight patients with abnormally high ammonia levels (of over 1  $\mu\text{gm}$  per ml), a significant fall occurred after amine oxidase inhibition. They deduced that degradation of amines might be partly responsible for the elevation of  $\text{NH}_4$  in liver disease. They observed that, in spite of reduction of ammonia, there was no clinical improvement in their cases. On the contrary one case sank into coma, and another had no regression of E.E.G. features of impending coma despite reduction of ammonia from 3.8 to 2.7  $\mu\text{gm}$  per ml.

They suggested that circulating amines may be more toxic to the brain than ammonium ions, or that unknown effects of the drug may be detrimental.

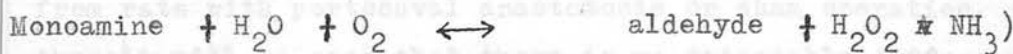


ACTIVITY OF MONO AMINE OXIDASE IN RAT BRAIN HOMOGENATES  
BY RATE OF DISAPPEARANCE OF SUBSTRATE 5-HYDROXY-TRYPTAMINE

Fig. 61.



It seemed possible that the metabolism of monoamines by the mono-amine oxidase reaction might be repressed by the presence of an excess of ammonium ions.



MAO

This prompted the study of the enzyme's activity by the most exact method available locally. It also brought up the question of the adaptation likely to occur if the enzyme were inhibited over a prolonged period. Might this underly reactive changes in astrocytes?

Two studies followed:-

- (1) Measurement of Amine Oxidase Activity in cerebral homogenate.
- (11) Histological Study of the Effects on the Brain of available Continued suppression of Amine Oxidase Activity with Iproniazid.

#### MONO-AMINE OXIDASE ACTIVITY

The essence of the method was the measurement of the rate of disappearance of 5 hydroxytryptamine, added in excess to an incubating homogenate of brain. 5 hydroxytryptamine was extracted from the samples by an ion exchange method of Crawford, Ashcroft and Ecclestone (modified from Uderfriend et al) and assayed by spectrofluorimetry.

The hypothalamic area was chosen for sampling because, according to the histochemical work of Shimizu (1959) the highest concentration of the enzyme occurs there. Least quantities occur in white matter, so, by dissecting out a block of tissue weighing approximately 20 mg. bounded on the inferior surface of the brain by the optic chiasm and tracts, and the cerebral peduncles, the hypothalamus with some central nuclear tissue was removed, and a minimum of white matter was included.

The results are expressed in the four graphs on Fig. 61.

Experiments 1 and 3 represent values obtained from four normal rats. They show how the substrate decays with time and that the values are repeatable. Experiments 2 and 4 show results from rats with portocaval anastomosis or sham operation. From these it will be seen that there is no detectable difference within the limitations of the experimental technique between animals with or without porto-caval shunting.

Dr. D. Ecclestone measured the concentrations of tryptamine and tryptophol in the samples, by thin layer chromatography, but no difference from normal was observed.

#### EFFECTS OF PROLONGED INHIBITION OF MONO-AMINE OXIDASE

The reduction of activity of mono-amine oxidase in the body as a whole can be achieved to 100% by exhibition of some of the potent inhibitors. Iproniazid was chosen because figures were available in the literature (Pletscher et al 1962) covering a range of inhibition values. If inhibition of 100% is maintained for more than a few days death occurs with massive yellow atrophy of the liver. 50% inhibition is tolerated indefinitely.

The dosage required for 50% inhibition from 16 - 30 hours by iproniazid was 1 mg. per kg. or 0.04 mg per 40 G animal. Mice were chosen for this part of the work rather than rats because of expense. It was easier to acquire a dozen mice of the same age and weight, and because tritiated thymidine was to be used to label any dividing glial cells, the smaller dose required for the mouse increased its appeal. It was realised that the use of a different animal was not desirable, but because of the pilot nature of the experiment no real objection was sustained.

Iproniazid was injected intraperitoneally into mice of about 40 G. The dose was repeated thrice weekly. Cumulative effects were detected by the appearance of bile-stained urine. When this occurred the next dose was delayed until the urine colour

had reverted to normal.

Animals were killed in pairs, weekly after the 12th week of treatment, and 12 hours after intracerebral injection of 10  $\mu$ c tritiated thymidine (this was done by Dr. W.E. Watson). Sections of brain were prepared for autoradiography. No morphological abnormalities were seen in the brain and no uptake of thymidine was observed.

#### DISCUSSION

Interference with the activity of amine oxidase in animals with porto-caval anastomosis was thought possible on theoretical grounds. No change was found by the assay method used, and the experiment was disbanded.

It was considered that, although the method was a tried and proven one, the sensitivity for such small quantities of brain (20 mg.) might be inadequate. It might have been more appropriate to have assayed the concentrations of other intermediary substances on the pathway, apart from tryptophol and tryptamine.

It might be that there is, in fact, no alteration of the activity of monoamine oxidase after porto-caval anastomosis.

The possibility that adaptation to inhibition of amine oxidase might be associated with the astrocytic response in liver disease was not borne out by the 50% inhibition study. the absence of glial response after three months is interesting in view of the work of Palmer of Cambridge on dogs. He found that prolonged exposure to these drugs caused reactive changes in protoplasmic astrocytes in most areas of grey matter. It might be that there is a difference in the rodent's metabolism which diminishes the significance of inhibition. It might also be that 50% inhibition is not sufficient to cause any important

compensation. There may be sufficient "biological excess" to make the 50% inhibition insignificant for the animal, or there may be alternative pathways for excesses of amines.

It might be that there is no adaptation by the rodent's neuro-glial tissue to amine oxidase inhibition.

#### SUMMARY

A diminution of amine oxidase activity was postulated on theoretical grounds to be possible in the animal with porto-caval anastomosis.

Measurement of mono-amine oxidase activity in rats with porto-caval anastomosis failed to demonstrate a difference from normal animals.

Prolonged inhibition of monoamine oxidase with Iproniazid was not followed by any demonstrable change in the histology of the brain of mice, and no uptake of tritium-labelled thymidine was demonstrated.

#### CONCLUSION

No evidence for the implication of mono-amine oxidase in the cerebral responses to liver disease has been found in rats with porto-caval anastomosis.



METHOD FOR THE ESTIMATION OF MONOAMINE OXIDASE IN BRAIN HOMOGENATES  
BY MEASURING THE RATE OF DISAPPEARANCE OF 5-HYDROXYTRYPTAMINE

1. Dissection of Brain

The animals were killed by a blow on the head, and the skull rapidly opened with nail clippers. The brain was removed intact. A small cube of tissue approximately 2 mm. deep, with surface boundaries of the optic tracts and chiasm, and the cerebral peduncles was removed quickly and carefully. Such a block contained the hypothalamus and part of the central nuclear mass, with a minimum of white matter. This was an area known to contain moderate quantities of the enzyme (Shimizu).

2. Homogenisation

The cube of brain was placed in an ice-cold, ground-glass, hand-operated homogeniser, with a small quantity of 0.3 M. Ammonium Acetate buffer. The volume of the homogenate was brought to 0.5 ml. with buffer. The homogenate was then transferred to a shaking incubator at 30°C. for 15 minutes, before the volume of standard serotonin creatinine sulphate solution was added. 2  $\mu$ g. 5 HT was added in these experiments.

At set time intervals 0.1. ml. of the medium was removed. The reaction was terminated by mixing with 0.5 ml. 4M.  $\text{HClO}_3$  and 4.4. ml.  $\text{H}_2\text{O}$ , giving a final volume of 6 ml. after the addition of 1 ml.  $\text{NH}_4$  Acetate buffer. The pH was adjusted to 7.5. with KOH, and the precipitate of  $\text{KClO}_3$  allowed to settle before adding the supernatant to the columns.

The columns contained a 70 x 4 mm. column of Amberlite CG 50 No. 1, in 0.3M.  $\text{NH}_4$  Acetate buffer.

The homogenate is allowed to drop through the column for 30 - 40 minutes.

The following sequence was then followed:-



1. Wash with 15 ml. 0.3 M  $\text{NH}_4\text{Ac}$ . buffer of pH 7.5. per 30 minutes.

2. Wash with 4 ml. 0.1  $\text{NH}_2\text{SO}_4$  for 10 minutes.

3. Wash with 6 ml. N.  $\text{H}_2\text{SO}_4$  and collect.

To the final eluate, containing the 5HT, 3 ml. conc. HCl. (11,419) with ascorbic acid is added immediately before reading. If delay was unavoidable between elution and reading, the samples could be stored overnight in a refrigerator, and the acid only added before reading.

A Beckmann spectrofluorimeter was used. Readings were made with an input excitation frequency of 400 and emission recording frequency of 450

RECEIVED  
RECEIVED  
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CHANGES IN WEIGHT AND STRUCTURE OF:-

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SPLEEN

HEART

KIDNEY

ADRENALS

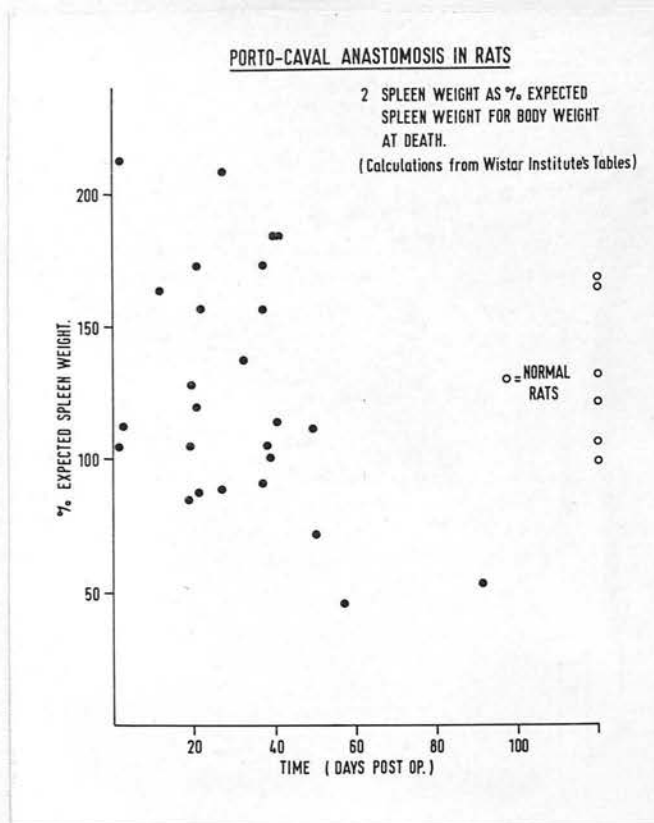


Fig. 62

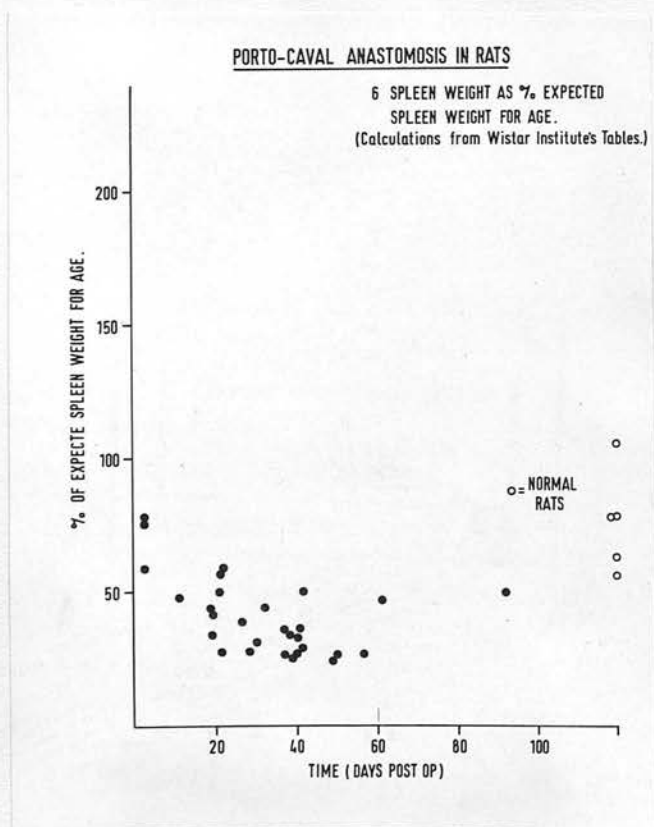


Fig. 63.

SPLEEN

At autopsy the spleens were dissected and weighed. Figs. 62 & 63 show the figures so obtained plotted as ordinates against time in days following the operation.

The spleen weights taken as a percentage of body weight at death show that the spleen is slightly smaller than in normal rats. Correction for the low body weight, by comparing the spleen's weight with either the expected spleen or body weight for the age of the animal emphasises this reduction.

In no case was there considerable splenomegaly. This was in accord with the absence of evidence of anastomotic stenosis, collateral portal-systemic drainage, or re-portalisation of the liver. These factors would tend to elevate the portal pressure, and such an eventuality could be associated with splenomegaly.

No recordings of pressure in the portal system were performed. In larger animals - e.g. dog, man - the portal pressure is 7 x 13.6 mm. H<sub>2</sub>O. The mean inferior vena caval pressure is 5 mm. H<sub>2</sub>O. After porto-caval anastomosis, abnormally elevated portal venous pressures fall to the vena caval pressure. The same must happen in the normal animal subjected to porto-caval anastomosis. The reduction of portal venous pressure may be the reason for diminution of splenic weight after shunting.

Two animals (rats 19 and 20) had pyogenic inflammations (bronchopneumonia and ulcerating sores) for some weeks before death. In these the spleens were also below normal.

H and E sections were prepared of all spleens but no abnormality could be seen in any. The relative proportions of lymphoid, granulocytic and erythrocytic cells seemed normal, but no attempt to quantitate was made.

Autoradiographic preparations were studied in 6 test and 3 control animals. No obvious difference in the DNA synthetic activity was detected in the two groups.



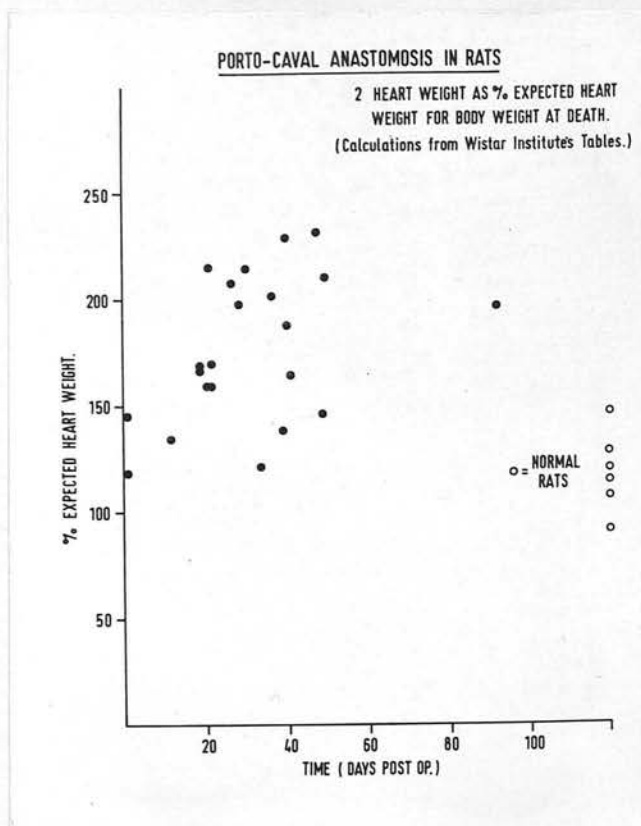


Fig. 64

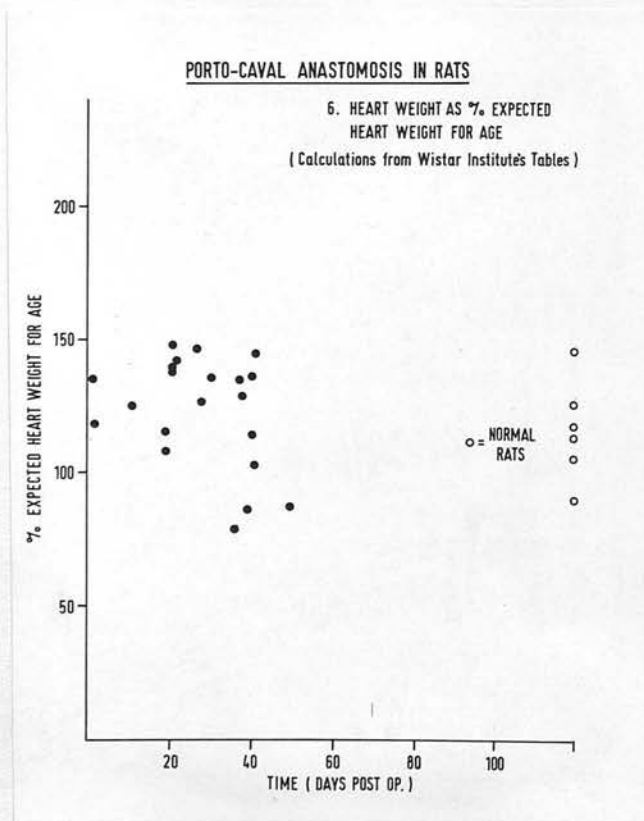


Fig. 65.



### HEART

At autopsy the heart was removed, the great vessels cut off at the atrial level, and the chambers opened to allow blood to be removed by saline washing.

The weights of the hearts were plotted as ordinates against the length of time following the operation. Figs. 64 & 65 are the resultant graphs. The expression of the heart weight in terms of the body's weight at death, appears to show increased cardiac mass. Correction for the loss of body weight by comparing the body weights and cardiac weights expected for the animal's age show that no change is demonstrable by these figures.

The heart appears to maintain its size in spite of falling body weight.

At least one H. & E. section of the ventricles was prepared from each animal. No abnormality of the muscle fibres, connective tissue, endothelium or quantity of fat was detected. The number of interfascicular nuclei did not appear to be increased in these preparations.

### KIDNEYS

The kidneys were removed at autopsy, cleared of fat, and weighed.

The total kidney weight (addition of both kidneys) was taken as a percentage of the body weight at death. This calculation shows that the percentage in rats with porto-caval anastomosis was greater than for normal rats. Comparison of the kidney weights with the expected body weight or total kidney weight for the animals' ages showed no difference from the normal range.

These figures show that the kidney mass is maintained despite the loss of body weight (Figs. 66 - 69).

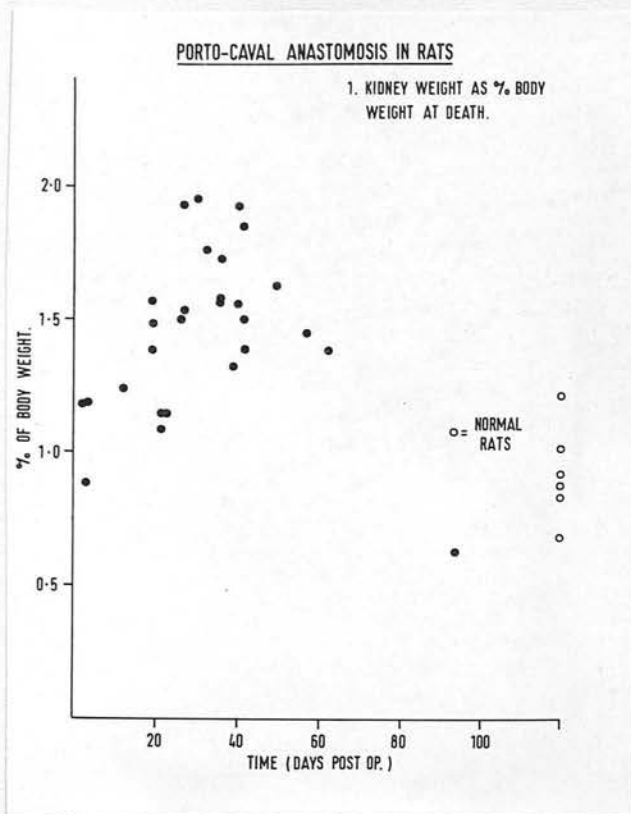


Fig. 66

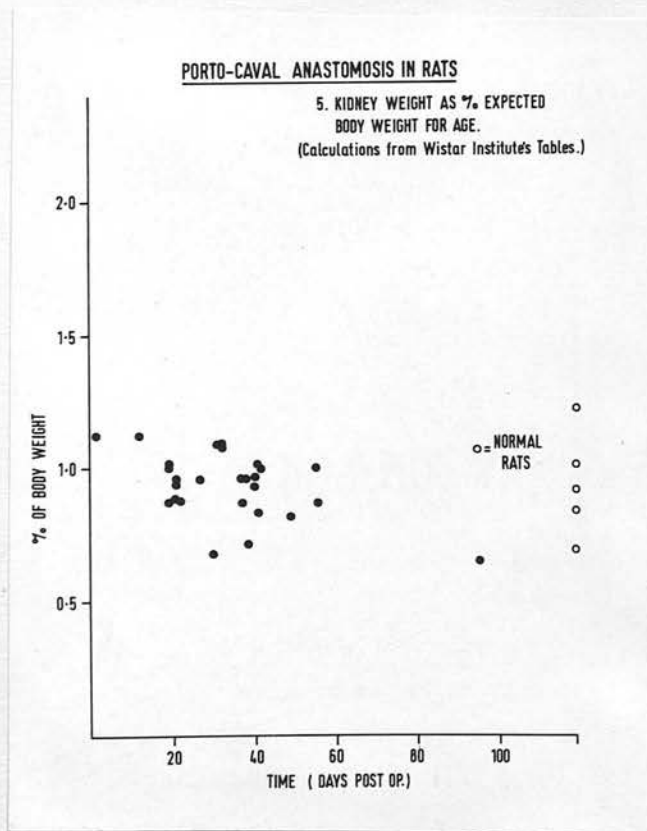


Fig. 67.

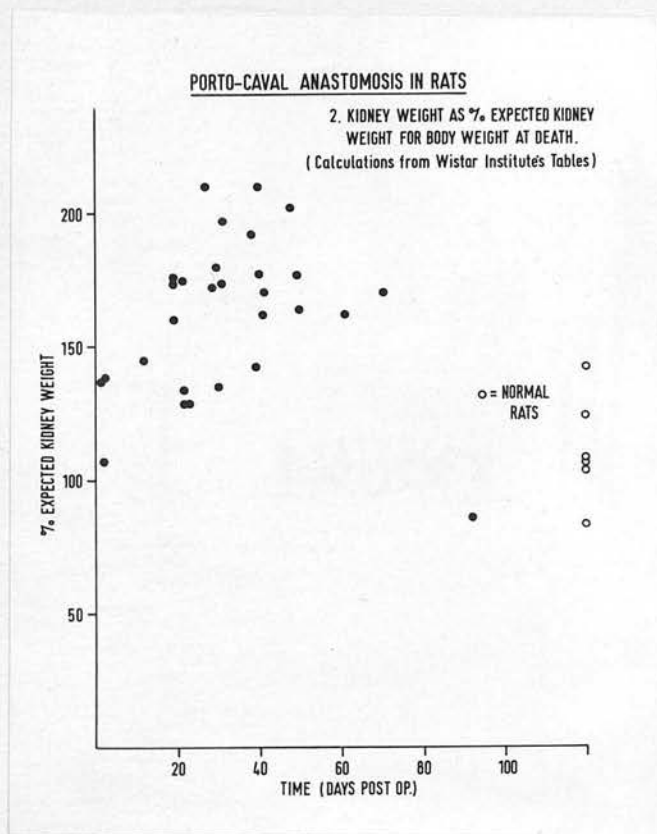


Fig. 68

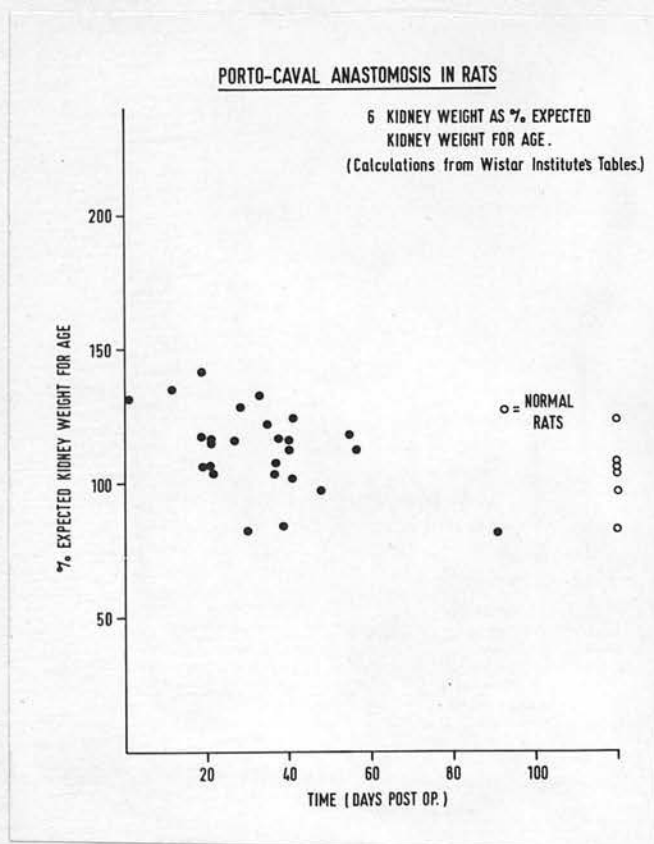


Fig. 69

Fig. 70  
(H.E. X 400)

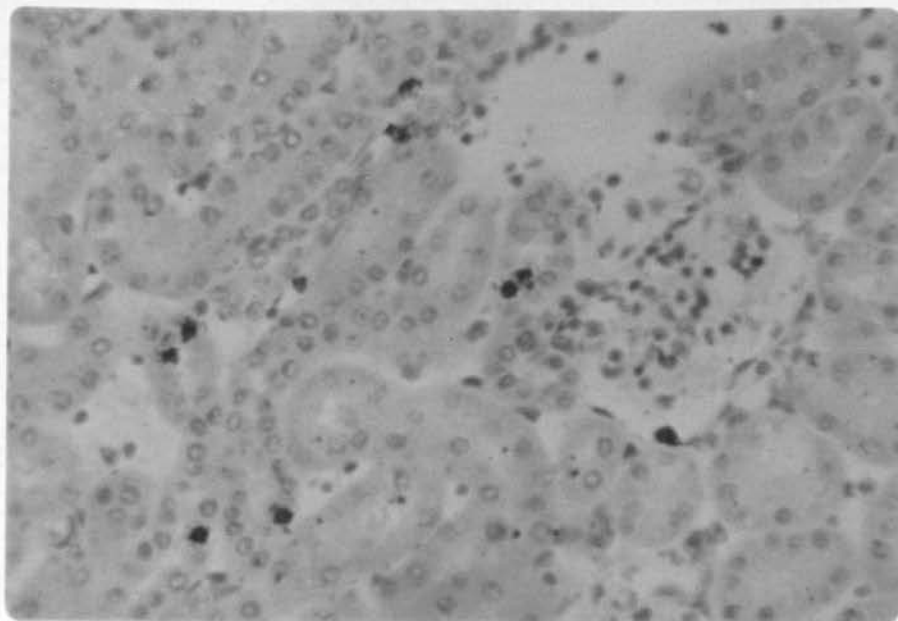
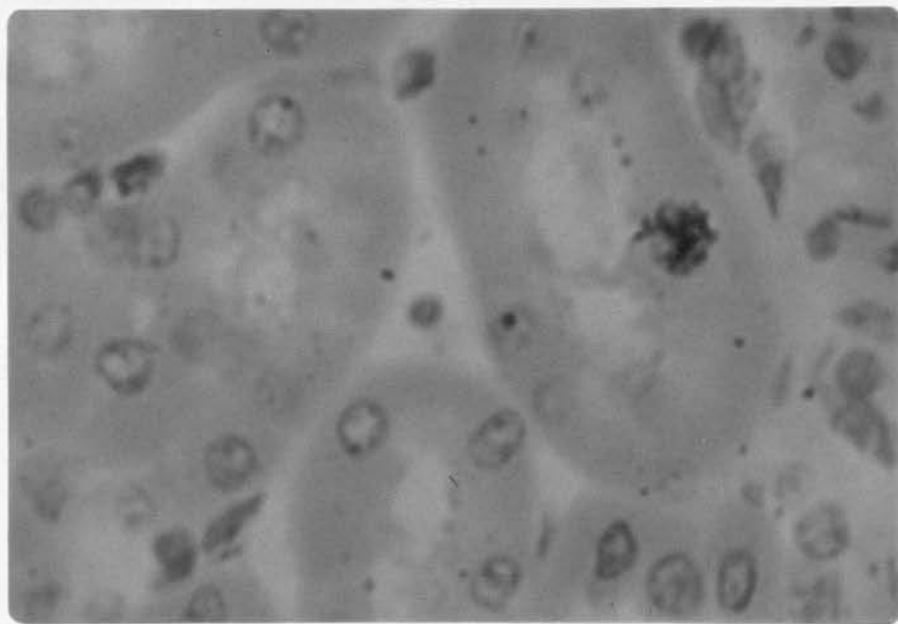


Fig. 71  
(H.E. X 1200)



Tritium-labelled thymidine uptake by the nuclei of kidney tubule cells, 56 days after porto-caval anastomosis. The interval between giving thymidine and death was 12 hours.



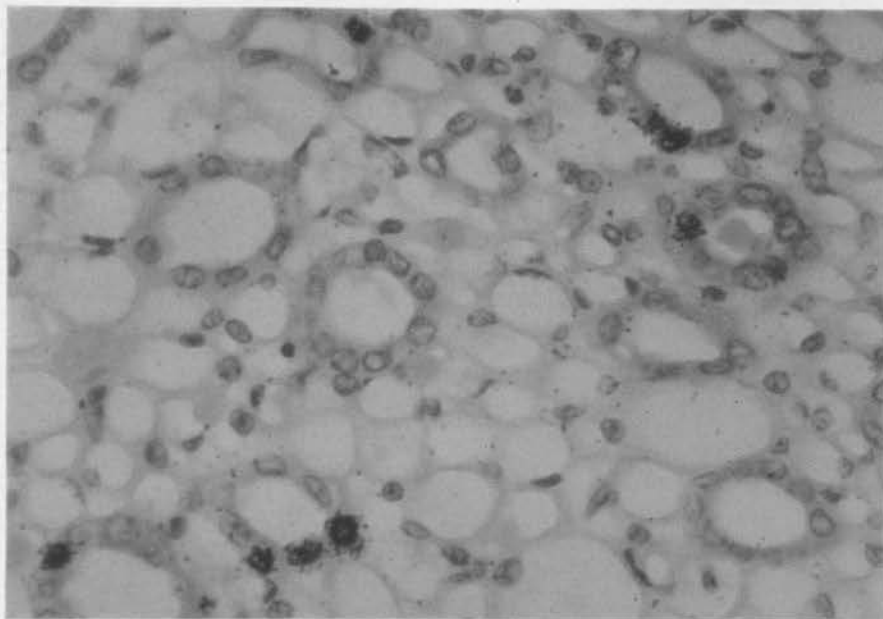


Fig. 72 Tritiated thymidine uptake by nuclei of kidney tubule cells 100 days after porto-caval anastomosis. The interval between giving thymidine and death was 12 hours.



Histological examination of thin sections - 4 u or less - revealed normal structure at all times after the operation. There was never evidence of cortical or tubular necrosis, such as could have happened in a prolonged operation where the systemic arterial pressure was reduced. Occasionally mild cloudy swelling and irregularity of the luminal surfaces of tubular cells were observed. This is a feature of non-specific electrolyte imbalance, especially of K depletion (M.McDonald). The glomeruli were always normal.

Autoradiographic preparations with  $^3\text{H}$ -Thymidine showed active DNA synthesis by cells of the tubular epithelium. This occurred in thick and thin limbs of the tubules (Figs. 70 - 72). The presence of numerous double-labels - i.e. labelled adjacent nuclei - suggests that division had actually occurred between the time of supplying the thymidine to the cells and killing the animal. The significance of such findings in kidneys showing no feature of abnormality by ordinary histology is difficult to postulate.

The number of labelled nuclei in several sections from the same kidney were counted, and the average number per section calculated. This was done in six rats with porto-caval anastomosis and three sham-operated controls of similar age, weight and sex.

The sham-operated rats had average counts of 20, 15, 7 per section at 34, 58, 82 days after operation.

The anastomosed rats had average counts of 100, 51, 104, 70 and 400 per section at 21, 21, 51, 95 and 220 days respectively.

There are insufficient figures to comment seriously about trends, but it is suggested that the number of labelled nuclei, i.e., cells preparing for division is higher in rats after porto-caval anastomosis than in sham-operated rats. The number of cells preparing for division may increase with the time after

# PORTO-CAVAL ANASTOMOSIS IN RATS

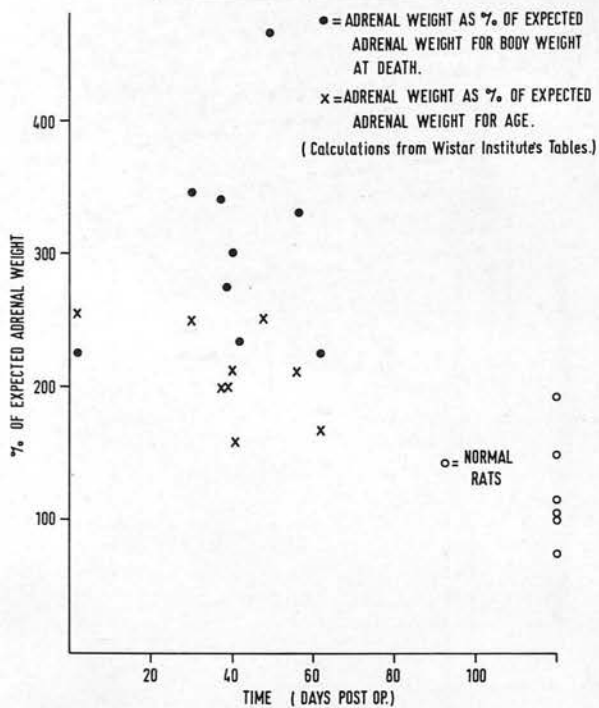


Fig. 73.

# PORTO-CAVAL ANASTOMOSIS IN RATS

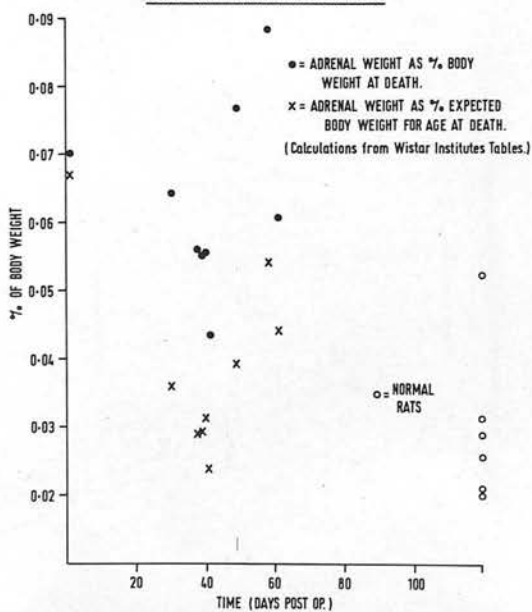


Fig. 74.

shunting. The rate of DNA synthesis in the rat surviving 200 days is very high.

Pyelonephritis was not encountered in this series of rats. This is a disorder said to occur frequently in colonies of old rats. It was probably related to the short survival of the animals in this series that it was not a complicating feature.

#### ADRENALS (Figs. 73 & 74)

The adrenal glands taken at autopsy and weighed were examined histologically. No abnormalities were observed and no difference between normal, sham-operated and test rats was apparent. There was no difference in the rate of incorporation of thymidine into nuclei.

Calculations were made as for the other organs to establish whether there were any change of adrenal mass. The glands were found to be slightly heavier on average than expected. The figures were too few for proper evaluation.

SUMMARY OF STUDY OF LIVER AFTER  
PORTOCAVAL ANASTOMOSIS IN RATS.

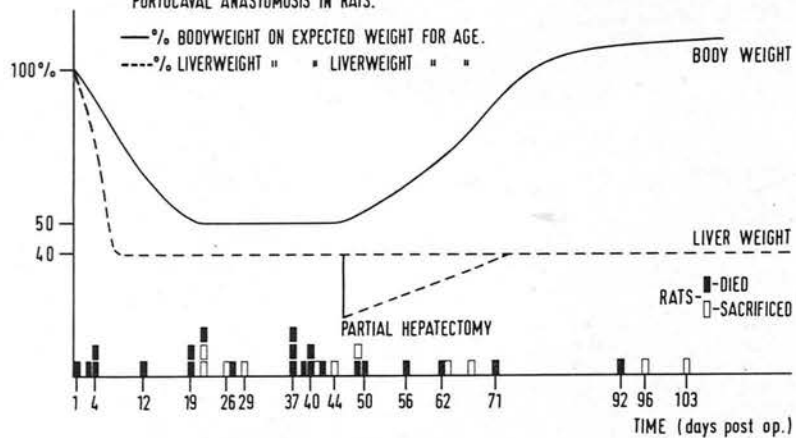


Fig. 75

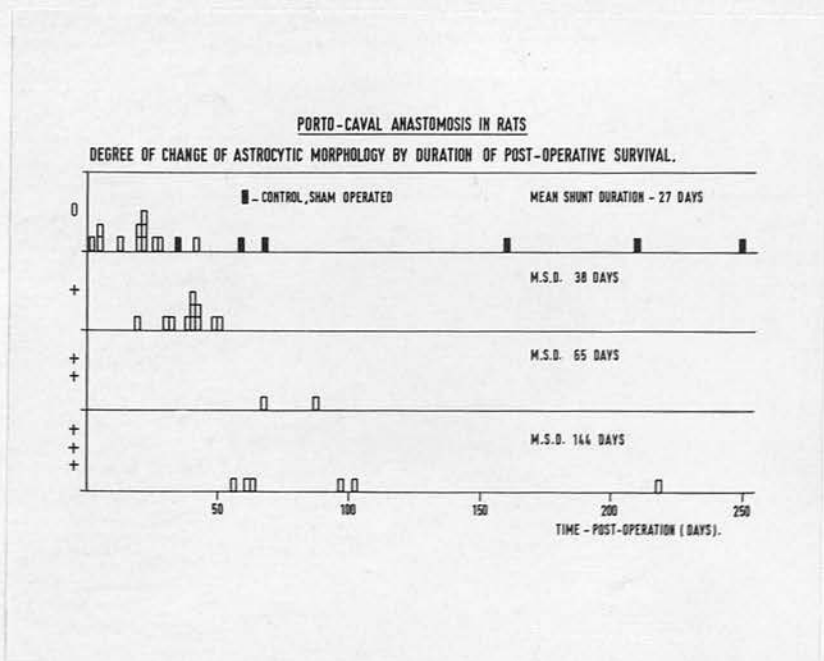


Fig. 76

0 and + represents a range of normal appearances of oligo-astrocytic nuclei in rats' brains, based on shape and staining properties.

++ and +++ are degrees of irregularity, both to the severity of changes in individual nuclei, and proportionately to the total number of oligo-astrocytes.

Fig. 76 (above) shows that morphologic changes occur only in rats with porto-caaval anastomosis, and that these changes are seen after 50 days survival.

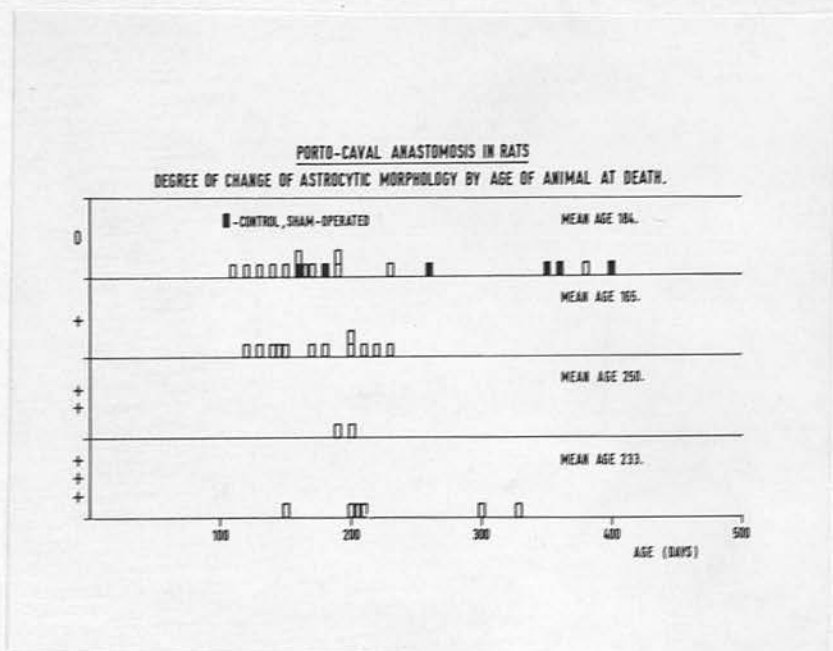


Fig. 77 shows that these changes are not a function of advancing age, because there are several operated and sham-operated animals, without changes in the oligo-astrocytes, of the same age or older than those with definite changes.



# CONCLUSIONS AND SUMMARY OF CHANGES IN RATS AFTER PORTO-CAVAL ANASTOMOSIS

A number of changes have been shown to occur in an orderly sequence after porto-caval anastomosis (Fig. 75).

A striking change occurs in body weight. This falls during the first 20 - 30 days after anastomosis. If the animal survives, a further period of 20 - 30 days of weight stability ensue, and thereafter there is a tendency for body weight to recover. The constancy of this pattern of behaviour suggests that the three phases could be labelled decompensation, balance and recovery.

During the phase of decompensation the liver weight drops to approximately 40% of the expected value and remains at the new proportion to body weight in spite of the phase of recovery.

Plasma ammonium concentration is elevated after shunting, but the level does not increase with time.

During the phases of decompensation and stability, no changes in the nervous system have been detected. After 55 days, morphological changes in astrocytes occur, and the changes appear to become more prominent with the progression of time (Figs. 76 & 77). These morphological changes are identical with the astrocytic changes seen in some forms of liver disease and in Wilson's disease.

Throughout the course of the animal's post operative life there is an increased rate of DNA synthesis by, and division of renal tubular cells, without significant accretion of kidney mass. The rate of turnover of tubular cells probably increases as time proceeds after anastomosis.

Throughout the course of the animal's post-operative life, but particularly in the first 50 days, there is a liability to haemorrhage from gastric erosions. No chronic gastric mucosal lesions have been demonstrated.

No evidence for the implication of cerebral monoamine oxidase in the pathogenesis of hepatic encephalopathy was sustained.

The splenic mass is reduced after porto-caval anastomosis.

### SUGGESTIONS FOR FURTHER WORK

The rat with porto-caval anastomosis is a very suitable model for studying the consequences of this procedure. Its applications are countless in furthering knowledge of this situation in man, and for unravelling some of the unknown effects of severe liver disease with compensatory shunting.

Pointers emerge from this work for advancement of knowledge of;

- (i) Brain pathology - The biochemical lesions underlying disordered astrocytic morphology.
- (ii) Brain physiology - evidence could be gained for the functions of glial elements.
- (iii) Gastric physiology - determination of the presence of a secretagogue other than histamine may be achieved.
  - evaluation of mucosal enzyme activity, e.g., histaminase, and histidine decarboxylase might be valuable.
- (iv) Renal pathology - There may be a clue to the functional relationships between the liver and kidney and the "hepato-renal syndrome".

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Thesis work can rarely be the work of one man working alone. No matter how much effort I may feel I have exerted single-handedly and single-mindedly on this work I have many to thank for many things.

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Dr. J.M. Walshe, Dept. of Experimental Medicine, Cambridge.

Professor S. Sherlock, Dept. of Medicine, Royal Free Hospital, London.

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Much of what is written in acknowledgement has not been constantly in my thoughts, but comes to mind readily when I start to set down this work.



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